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# CONTENTS

# A KINETIC STUDY OF THE PERIODATE ION AND THE PERIODATE-GLYCOL COMPLEX *

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The theory of specific oxidation of glycols by coordinating oxidants[1,2] provides a method for studying the coordination chemistry of such oxidants. The present paper illustrates the application of this method in the simplest case to which it may be applied, the periodate ion. This ion is generally considered to have a coordination number of two, a conclusion verified using this kinetic method.

It has been shown that periodate oxidizes glycol through the disproportionation of a periodate-glycol complex, and that in .008 M periodate and 0.2 M glycol at $0°$ C., essentially all of the periodate exists in the form of the glycol complex. If the glycol concentration is increased beyond this point, three possibilities exist: 1. The coordination number of periodate ion may be greater than two, allowing coordination of more than one glycol molecule; or 2. The complex containing one glycol molecule might react with another glycol according to reaction 1.

$$1.\ O_4I \begin{matrix} HOCH_2 \\ | \\ HOCH_2 \\ C_1 \end{matrix} + \begin{matrix} CH_2OH \\ | \\ CH_2OH \end{matrix} \rightleftharpoons O_4I \begin{matrix} HOCH_2CH_2OH \\ \\ HOCH_2CH_2OH \\ C_2 \end{matrix}$$
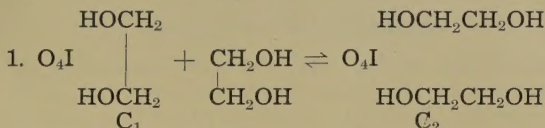
3. The periodate ion may react to form $C_1$ only.

## THEORY

In glycol concentrations above 0.2 M, the unglycolated periodate ion essentially disappears (1), leaving total periodate, (A) in any form expressible by the following equation:

$$(A) = (C_1) + (C_2)$$

where $C_2$ may be as in reaction 1 or may have both glycols coordinated through both oxygens. In either case, the kinetics is expressible in the form[2]

$$\frac{-d(A)}{dt} = k_1(C_1) + k_2(C_2).$$

The general theory would predict that the complex containing only

[1] Duke, Jour. Amer. Chem. Soc., 69, 3054 (1947).
[2] Ibid, *69*, 2885 (1947).

glycols coordinated through one oxygen would not disproportionate and $k_2$ would be equal to zero. For this special case, then,

$$\frac{-d(A)}{dt} = k_1 C_1 \text{ and } K_2 = \frac{(C_2)}{(C_1)(G)}, \text{ where (G) is the glycol concen-}$$

tration. Solving for $C_1$ in terms of $(A)$,[2] we obtain

$$\text{I.} \quad \frac{-d(A)}{dt} = \left[\frac{k_1}{1 + K_2(G)}\right](A)$$

If $C_2$ may also disproportionate,

$$\text{II.} \quad \frac{-d(A)}{dt} = k_1 C_1 + k_2 C_2 = \left[\frac{k_1 + k_2 K_2(G)}{1 + K_2(G)}\right](A)$$

If $(G)$ is maintained very high compared with $(A)$, the reaction is pseudo-first order in $(A)$, and pseudo constants, $k'$, may be determined experimentally, $k'$ being identified with the bracketed portion of I or II. From $k'$ at several glycol concentrations $k_1$, $k_2$, and $K_2$ may then be determined.

### EXPERIMENTAL

The experiments were done at $0°C.$ in the same manner as described in reference 1, except that a solution of sodium periodate containing the equivalent amount of nitric acid was used instead of periodic acid.

### RESULTS AND DISCUSSION

The slopes of plots of log $(A)$ vs $t$ were measured. Good straight lines were obtained in each case; these are tabulated as $k'$ at various glycol concentrations (Table 1). It will be noted that there is very little variation in $k'$ in going from a $(G)$ of 0.2 to 2.0 M, though, in general, there is a slight regular increase in rate with increasing concentration which becomes noticeable above 2.0 M glycol. It is in this range that the glycol concentration becomes high enough to significantly change the character of the solvent. It will be noted that the addition of alcohol has much the same effect. Thus we conclude that $k'$ does not change due to change in the primary complex.

Applying equation I to these results leads to the conclusion that $K_2$ is zero or exceedingly small: $\frac{-d(A)}{dt} = k_1(A)$. Thus, a doubly glycoted complex containing each glycol molecule coordinated through a single oxygen is eliminated.

Equation II will allow constancy of $k'$ only under one of two conditions: (1) $K_2$ is equal to zero leading to $\frac{-d(A)}{dt} = k_1(A)$; or (2) $k_2 = k_1$, leading to the same expression. The likelihood of the latter seems exceed-

## TABLE 1

VARIATION IN K′ WITH [G]

| [G], M/1 | k′ x $10^3$ | [ G ], M/1 | k′ x $10^3$ |
|---|---|---|---|
| .05 | 1.50 | 1.3 | 2.07 |
| .1 | 1.70 | 1.6 | 2.03 |
| .2 | 1.99 | 2.0 | 2.19 |
| .6 | 2.07 | 2.4 | 2.20 |
| 1.0 | 2.08 | 3.0 | 2.28 |

ingly small, especially in the light of the extreme differences between $k_1$ and $k_2$ in a comparable case, the disproportionation of mono and di oxalato manganiate[2,3] and the differences observed in the cerate com-plexes of bulylene glycol.[4] The evidence points to the conclusion that periodate ion has a maximum coordination number of two.

[3] Taube, Jour. Amer. Chem. Soc., 70, 1216 (1948).
[4] Duke and Forist, Jour. Amer. Chem. Soc., 71, 2790 (1949).

# SEEDS OF AGRICULTURAL AND WEEDY *BROMUS*[1]

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The seeds of several species of *Bromus* are common contaminants of forage grass and small grain seed. One of these species, chess (*Bromus secalinus*), is declared a noxious weed by a number of states; its seeds are frequent among those of small grains as well as forage grasses. The identification of incidental seeds in connection with the purity analysis of crop seeds necessitates continuous reference to seeds of weedy species of *Bromus*. Such identifications may be critical in regard to the monetary value of seed lots when they relate to the distinction between seeds of chess and those of related species. Additionally, the seed technologist must be familiar with the seeds of cultivated species of *Bromus* (*Bromus inermis, B. catharticus,* and *B. carinatus*) so that purity determinations may be accurately carried out.

Seeds and seed-like structures frequently possess distinctive characteristics which are of taxonomic value in properly classifying or distinguishing between related species. Such characters, although usually not included in most plant identification manuals, may be particularly valuable to botanists in identifying fragmentary or out-of-season material. For example, tentative identification of *Bromus* specimens may frequently be verified or corrected through examination of the seed.

Literature concerning brome seeds consists primarily of short notes and illustrations of seeds of some species [Beal (1); Hillman (3); Hillman and Henry (4); Roberts and Freeman (8)]. The present publication is designed to make pertinent information regarding the economic species of bromes available to seed technologists and botanists. The present publication describes the seeds of twelve species of *Bromus* which possess agricultural interest as crop plants, range species, or weeds. Although there are more than 30 species of *Bromus* in the United States, most of these have little application to agriculture and are not considered in the present study.

## DISTRIBUTION AND RELATIONSHIPS

Following the interpretation of Hitchcock (5), the species treated in this paper represent members of four different sections of the genus *Bromus*. These are *Ceratochloa, Bromopsis, Bromium,* and *Eubromus*.

*Ceratochloa,* consisting of American species with strongly compressed

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spikelets, includes *Bromus carinatus* and *B. catharticus.* The former is native to western North America; the latter to Argentina. *Bromopsis* includes perennial species native to both Eurasia and North America. *Bromus inermis*, originally native to Europe, belongs to this group. *Bromium* is characterized by annual Eurasian species with broad lemmas and spikelets. Representatives of this section included in the present study are *Bromus racemosus, B. mollis, B. secalinus, B. commutatus,* and *B. japonicus.* These species are very closely related and their present taxonomic arrangement is not entirely satisfactory. *Eubromus* is represented by annual Eurasian species with narrow lemmas and spikelets. *Bromus rubens, B. tectorum, B. sterilis,* and *B. rigidus* belong to this group.

From the above summary it may be observed that only one of our economic species of *Bromus, B. carinatus,* is native. The remaining species, with the exception of *B. catharticus,* are all introduced from Eurasia. Most of these plants are now widely distributed in the United States.

## PROCEDURES

Many of the seeds employed in this study consisted of material removed from herbarium specimens. The identity of all such specimens was verified by the junior author, Dr. R. W. Pohl. In the difficult *Bromium* section (*Bromus secalinus* and relatives), conclusions drawn from herbarium seed material were checked with seeds found in agricultural seed samples analyzed in the Iowa State College Seed Laboratory and samples sent to the authors by other seed laboratories. Seeds from a number of these samples were planted in the greenhouse and (if viable) grown to maturity. This procedure served both to verify tentative identifications and to ascertain if progeny seeds were similar to those of the parent.

An ocular micrometer was used in making measurements of seeds, and was particularly valuable in studying palea-lemma relationships in *Bromus secalinus* and *commutatus.* Once the micrometer is calibrated for any given magnification, measurements to one-tenth of a millimeter can be easily and quickly made.

### GENERAL CHARACTERISTICS OF *BROMUS* SEEDS

The spikelets of *Bromus* species are relatively large, usually 1–3 cm. in length, and contain 3–12 flowers. The glumes are unequal in size, the second generally the larger. The lemmas are awned from between or below a pair of apical teeth, or are awnless; they possess 5–9 longitudinal nerves, but these are frequently weak and difficult to discern. At maturity the spikelet rachis disarticulates below the attachment of each lemma, and the individual caryopses together with their enveloping lemmas and paleas are freed from the parent plants. Such seed-like units, common to many grasses, are popularly called seeds, and for

simplicity will be so designated in this paper. Morphologically, they are not seeds, but are matured florets.

*Bromus* seeds (excluding the awn) range from 6 to 18 mm. in length. The grain or caryopsis is completely enclosed within the tightly appressed lemma and palea. The lemma, covering the embryo (dorsal) side of the grain, is usually considerably longer than both grain and palea. The palea, adherent to the ventral side of the grain, usually approximates the latter in length. The rachilla, representing the segment of spikelet rachis between points of disarticulation is usually present on *Bromus* seeds. It is attached at the base of the seed and lies against the palea face. Scars at the lower end of the seed and at the apex of the rachilla indicate points of abscission between successive florets.

The most striking differences between seeds of various *Bromus* species have to do with the shape. In a species such as *B. inermis* the grain is flat and dorso-ventrally compressed; the lemma and palea are flat. There is considerable variation in shape of the grain in the group of species allied to *B. secalinus* (*B. commutatus, japonicus, racemosus,* and *mollis*); such variation is both between different species and within a given individual species. The grain may be flat and thin, similar to that of *B. inermis,* or it may be laterally curved so that the ventral surface is concave and the dorsal surface is convex. The amount of this lateral curvature is extremely variable. The grain may be boat-shaped with a relatively open ventral surface; the lateral portions of the seed may ascend vertically; or the seed be abruptly folded with the edges closely contiguous. When the latter condition is approached, the ventral surface is hidden from view or nearly so, and the grain appears to be laterally compressed. The term *ventral furrow* will be subsequently employed in this paper to characterize the partially or completely enclosed ventral surfaces of such grains.

In *Bromus catharticus* a further modification of the grain has taken place. It is sharply folded longitudinally and the ventral surface enclosed. The ventral furrow, however, appears quite shallow. This is due to the fact that the adjoined halves of the ventral surface have become completely fused except for the marginal extensions. The result is a laterally compressed solid grain with a pair of longitudinal flanges running down the ventral edge.

Diagrammatic representations of cross sections of seeds of various species of *Bromus* are given in Fig. 17. The conformation of the surrounding lemma and palea as well as the shape of the grain is illustrated. Consideration of this variation in grain morphology, particularly the apparent sequence from a dorso-ventrally flattened to a folded, ventrally fused structure, may be of significance in interpreting phylogenetic interrelationships between various species of *Bromus.*

The over-all shape of the lemma is primarily dependent upon the form of the grain within. It ranges from nearly flat, to boat-shaped, to longitudinally folded with the lateral margins curving together. The

lemma is rather broad (ovate if spread out flat) in *B. secalinus* and related species, or quite long and narrow (lanceolate if spread out) in *B. tectorum* and its allies (*B. sterilis, rigidus,* and *rubens*). The basal portion is differentiated into a hard ring-like *callus* surrounding the scar at the lower end of the seed. The lemma is usually two-toothed at the apex; the length and character of these teeth differ markedly in different species. A terminal awn is present in most species. It represents a continuation of the midvein and departs from the body of the lemma at the base of the apical teeth. The awn is rudimentary or absent in *Bromus inermis* and *B. catharticus*. Owing to the destruction of the awn, seeds of other species sometimes also appear awnless.

The shape and appearance of the palea, like that of the lemma, is dependent upon the form of the grain. In *Bromus inermis, B. mollis,* and *B. racemosus* the palea is frequently nearly flat; in other species it is laterally curved, strongly concaved or sharply folded. Lying primarily within the ventral furrow, it is commonly either partially or completely hidden from external view. In length, this structure is usually subequal to the mature grain.

In most bromes the palea is slightly wider than the caryopsis, completely covering its ventral surface and folded around a short distance on the back (*see* Fig. 17). Along the margins of the caryopsis it possesses a longitudinal raised fold, the so-called marginal keel. This keel is characteristically ciliate, the nature and density of the hairs varying from species to species. In *Bromus inermis*, the keel is not marginal on mature seeds.

## ANALYTICAL KEYS
### KEY BASED ON SEED CHARACTERS

1. Apical teeth of lemma 2–5 mm. long; seeds long and slender, the length of the lemma usually 5–6 times maximum width; awn equaling or much exceeding lemma.
  2. Palea bristles capillary, various in length on same seed; palea usually visible externally; seeds 7–14 mm. in length exclusive of awns, thin, commonly somewhat backwardly bent; lemma (with infrequent exceptions) dorsally villous-pubescent.
    3. Lemma 7–11 mm. long exclusive of awn; grain boat-shaped; palea with a single medial ridge or fold; plants occurring over entire United States.
          *Bromus tectorum*
    3. Lemma 10–14 mm. long exclusive of awn; grain nearly flat; palea at maturity usually with 3 central ridges or folds; plants limited to Pacific and southwestern states.    *B. rubens*
  2. Palea bristles stiff, uniform in length; palea commonly concealed by incurved lemma; seeds 12–25 mm. in length exclusive of awns, stiff, straight; lemma scabrous-pubescent.
    4. Lemma, exclusive of awn, 20–25 mm. long; apical teeth 4–5 mm. in length; callus usually bearded over entire surface.    *B. rigidus*
    4. Lemma, exclusive of awn, 12–18 mm. in length, apical teeth 2–3 mm. long; callus glabrous except for marginal tufts of hair.    *B. sterilis*
1. Apical teeth of lemma 0.2–1.0 mm. long; seeds frequently relatively broad, the lemma usually 3–5 times longer than wide; awn commonly shorter than lemma.
  5. Palea bristles 0.2–0.4 mm. in length, not crowded on keels, spaced 0.1–0.3 mm. apart; lemma rather broad, 6–9 mm. in length; rachilla frequently enlarged upwards.
    6. Lemma short–hairy over entire surface (many of hairs may be destroyed on seeds occurring in agricultural seed); seeds usually thin and flattened.
          *B. mollis*
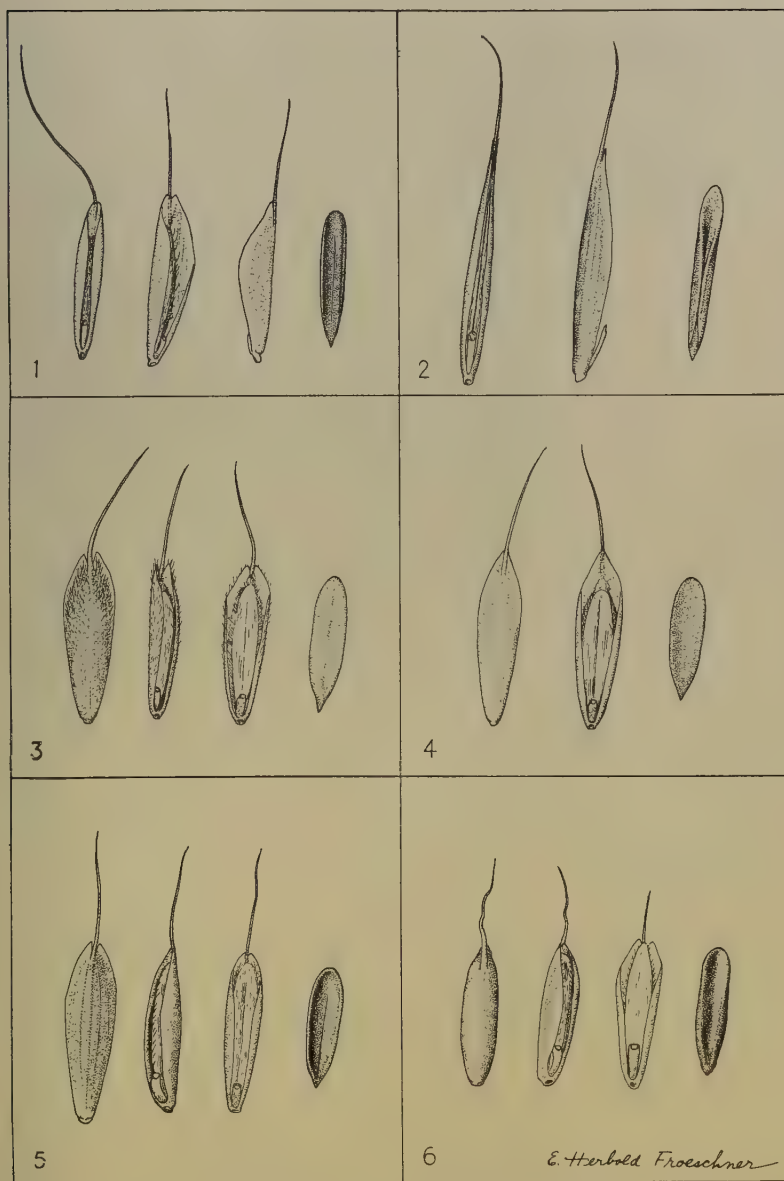
FIGS. 1–6.—1. *Bromus japonicus* Thunb.. 2. *B. carinatus* Hook & Arn. 3. *B. mollis* L. 4. *B. racemosus* L. 5. *B. commutatus* Shrad. 6. *B. secalinus* L. All x2½.

6. Lemma glabrous or marginally puberulent; seeds various in shape, usually not flattened.
  7. Apical scar of rachilla vertical; length of lemma normally exceeding palea and grain by 0.9–1.2 mm.; grain thin and fragile, usually strongly concave, (commonly with edges nearly contiguous on lower portion but somewhat flaring towards tip); awn commonly bent backwards.                    *B. japonicus*
  7. Apical scar of rachilla oblique; lemma subequal to palea and grain or exceeding it by as much as 0.9 mm.; grain various; awn various.
    8. Palea approximating lemma or slightly shorter (to 0.3 mm.); lemma (exclusive of awn) 6.0–7.8 mm in length (most seeds 6.8–7.5 mm.,averaging about 7.2); grain thick and heavy at maturity—immature grains thinner—usually folded with lateral margins contiguous; awn absent or present, 1–6 mm. in length, frequently with an abrupt bend near base.
                                                                *B. secalinus*[3]
    8. Palea 0.3–0.9 (1.5) mm. shorter than lemma; lemma 6.0–10 mm. in length (most seeds 7.4–8 mm.); grain thin or somewhat thickened, flat or folded longitudinally; awn present (commonly broken off), 5–10 mm. long, usually straight.
      9. Seeds boat-shaped, laterally folded, or less commonly flattened; lemma usually indistinctly 5–7 nerved, infrequently cross-wrinkled; palea not possessing a distinct medial, longitudinal seam; plants most common in eastern United States.                    *B. commutatus*
      9. Seeds flattened, less commonly boat-shaped or laterally folded; lemma frequently distinctly 5–7 nerved and cross-wrinkled; palea usually with a distinct medial longitudinal seam or fold; most common on Pacific coast.                    *B. racemosus*
5. Palea bristles 0.1 mm. in length or less, closely crowded on keels; lemma broad or narrow, 8–18 mm. in length; rachilla only slightly enlarged upwards.
  10. Seeds flat, 8–10 mm. in length; palea completely exposed, the keels not marginal on mature seeds.                    *B. inermis*
  10. Seeds boat-shaped or longitudinally folded, 10–18 mm. in length; palea partially or completely hidden within lemma and grain, keels marginal or nearly so.
    11. Seeds slender, awned; lemma glabrous or pubescent, weakly nerved.
                                                                *B. carinatus*
    11. Seeds broad, laterally flattened, awnless; lemma glabrous or finely hispidulous, strongly striate-nerved.                    *B. catharticus*

### KEY BASED ON PLANT CHARACTERS

1. First glume 1-nerved.
  2. Plants bearing rhizomes, perennial; lemmas with awns 1 mm. long or shorter; apical teeth of lemmas short (less than 1 mm. in length), acute.
                                                                *Bromus inermis*
  2. Plants lacking rhizomes, annual; lemmas with awns 5 mm. or more long, the apical teeth acuminate; 2–5 mm. in length.
    3. Panicles dense, cylindrical, the branches short, ascending, mostly concealed by the spikelets which are usually reddish at maturity.                    *B. rubens*
    3. Panicles open, the branches spreading or drooping; spikelets various in color.
      4. Panicle branches very slender and drooping, the spikelets pendant; second glume 10 mm. long or less; lemmas usually softly pubescent, bearing awns 1–2 cm. long.                    *B. tectorum*
      4. Panicle branches usually spreading or ascending, rather stiff; second glume 12 mm. or more long; lemmas scaberulous or stiff-pubescent.
        5. Awns 2–3 cm. long; second glume 12–15 mm. long.                    *B. sterilis*
        5. Awns 3–5 cm. long; second glume 22–27 mm. long.                    *B. rigidus*
1. First glume 3–5 nerved.
  6. Panicle open, pyramidal on well developed individuals, racemose on depauperate individuals; panicle axis exposed, branches spreading or drooping.
    7. Spikelets strongly flattened, mostly 2–3 cm. in length; lemmas and glumes V-shaped in cross section; lemmas exceeding 1 cm. in length.
      8. Lemmas awnless or with short awns 1–3 mm. long.                    *B. catharticus*
      8. Lemmas with well-developed awns 5–15 mm. long.                    *B. carinatus*

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[3] Seeds of this and the following species cannot always be clearly distinguished. See descriptions for further interpretation of diagnostic characters.
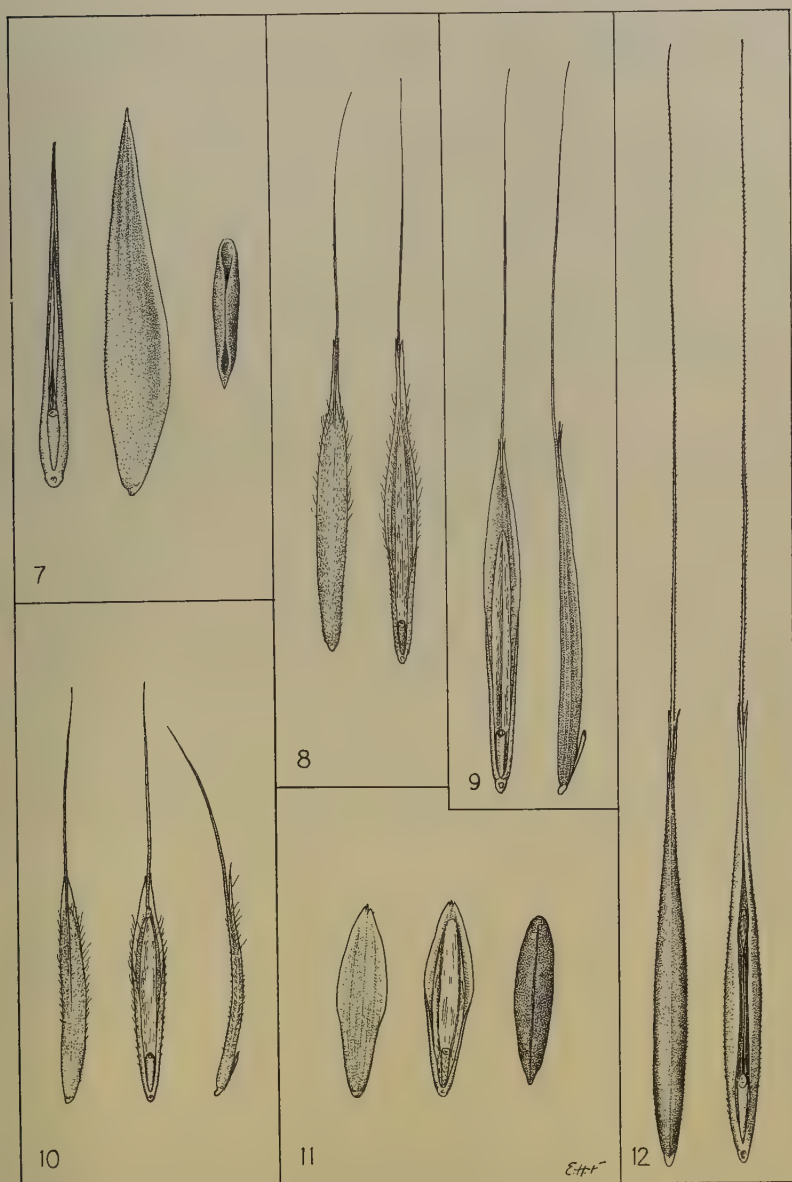
Figs. 7–12.—7. *Bromus catharticus* Vahl. 8. *B. rubens* L. 9. *B. sterilis* L. 10. *B. tectorum* L. 11. *B. inermis* Leyss. 12. *B. rigidus* Roth. All x2½.

7. Spikelets plump or slightly flattened, mostly 1–2 cm. in length; lemmas and
paleas curved in cross section; lemmas less than 1 cm. in length.
   9. Sheaths, except sometimes the lowermost, glabrous; spikelet rachilla
   usually exposed at maturity; lemmas often short-awned or awnless.
                                                              *B. secalinus*
   9. Sheaths hairy; spikelet rachilla not exposed at maturity; lemmas long-
   awned.
      10. Spikelets lanceolate, 3–5 mm. wide when mature; branches of panicle
      elongated, very slender and flexuous; lower sheaths densely covered
      with soft, white hairs which droop toward the ends and often become
      matted or tangled.                                     *B. japonicus*
      10. Spikelets narrowly ovate to oblong, 5–8 mm. wide when mature;
      branches of the panicle ascending to drooping at maturity, not flexuous;
      lower sheaths sparsely to rather densely covered with straight, spread-
      ing to retrorse white hairs.                          *B. commutatus*
6. Panicle ellipsoid, dense, usually 10 cm. or less long, the branches shorter
than the spikelets, which overlap and conceal the axis.
   11. Lemmas glabrous.                                      *B. racemosus*
   11. Lemmas pubescent.                              .       *B. mollis*

## DESCRIPTIONS OF SEEDS

**Bromus carinatus** Hook. and Arn. [ Incl. **B. marginatus** Nees., **B. polyan-
thus** Scribn., **B. maritimus** (Piper) Hitch.] Mountain Brome,
California brome.

(Figs. 2, 17b.)

OVER-ALL APPEARANCE. A keeled, stiff, slender seed with an awn.

LEMMA. 10–18 mm. in length, much exceeding the grain, laterally folded
so that the margins come together and frequently hide the grain and
palea from view. Dorsal surface glabrous or pubescent; midnerve form-
ing a dorsal keel. Apical teeth inconspicuous. Awn stout, 5–15 mm. in
length. Callus glabrous.

PALEA. Exceeding the grain but usually shorter than lemma, nearly
completely hidden from view by enclosing lemma and grain; keels
marginal, the bristles closely crowded, short, 0.1–0.2 mm. long.

RACHILLA. 2–3 mm. long, slender, of nearly same diameter at base and
apex, straight, glabrous or finely villous; scar vertical or nearly so.

GRAIN. 6–10 mm. long, appearing narrowly oblong and flattened, laterally
folded so that the edges are in close proximity, the ventral groove very
narrow or almost absent. (Fig. 17b).

DISCUSSION. *Bromus carinatus* is native from British Columbia to South
Dakota, southward to western Texas, northern Mexico, and California.

The plants representing this species are quite variable and are
sometimes regarded as several closely related species (note synonyms
above). It does not seem possible, however, to draw clearcut lines be-
tween the various forms included in this complex. Many of them are
probably only local forms perpetuated through seed set from self-
fertilized cleistogamous flowers [Harlan (2)].
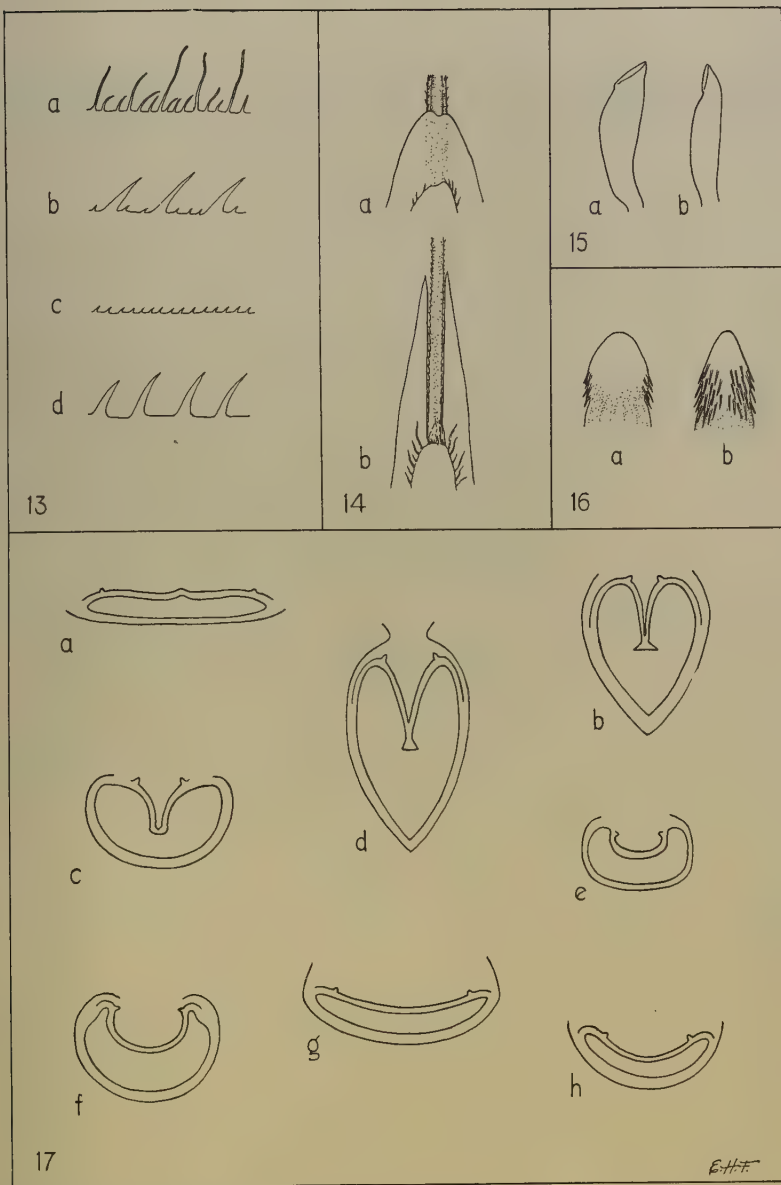
FIGS. 13–17.—13. Palea keel teeth. a. *Bromus tectorum* L. b. *B. rigidus* Roth. c. *B. inermis* Leyss. d. *B. commutatus* Shrad. 14. Apex of lemma. a. *Bromus commutatus* Shrad. b. *B. tectorum* L. 15. Apex of rachilla. a. *Bromus commutatus* Shrad. b. *B. japonicus* Thunb. 16. Callus. a. *Bromus sterilis* L. b. *B. rigidus* Roth. 17. Cross sections of seeds. a. *Bromus inermis* Leyss. b. *B. carinatus* Hook. & Arn. c. *B. sterilis* L. d. *B. catharticus* Vahl. e. *B. tectorum* L. f. *B. secalinus* L. g. *B. japonicus Thunb.* h. *B. commutatus* Shrad. Fig. 13, x17. Figs. 14–16, x14. Fig. 17, various.

Mountain brome has a high forage value and is employed in range land reseeding in the western Great Plains and Rocky Mountains. Limited commercial seed stocks are now available.

## Bromus catharticus Vahl. (B. unioloides H. B. K.) Rescue grass
(Figs. 7, 17d.)

OVER-ALL APPEARANCE. A large, awnless, laterally flattened seed with conspicuous nerves.

LEMMA. 12–18 mm. in length, longitudinally folded — the two sides nearly flat, closely pressed together, almost completely concealing palea and grain. Surface strongly 3–5 nerved on each side, the nerves and sometimes the inter-spaces finely hispidulous; midvein keel-like. Apical teeth very short, subtending a much reduced (0.5–1 mm. long) awn-like bristle. Callus glabrous or finely hairy.

PALEA. Approximately 1 cm. in length, enclosed within lemma; keels marginal, contiguous, the bristles very short and crowded.

RACHILLA. 2–4 mm. in length, slightly increasing in width to apex, straight or somewhat bowed, finely hispidulous; apical scar vertical.

GRAIN. Approximately 1 cm. in length, much exceeded by lemma, strongly compressed laterally; dorsal and ventral edges less than 1 mm. wide, sides 1–2 mm. high; ventral groove very narrow and extending only about ⅓ depth of the seed (Fig. 17d).

DISCUSSION. Rescue grass is grown in the southern states as a winter forage crop and volunteers freely, especially on low, moist ground. Under field conditions in this country, it behaves as a winter annual.

## Bromus commutatus Shrad. Hairy chess
(Figs. 5, 13d, 14a, 15a, 17h.)

OVER-ALL APPEARANCE. Thin, boat-shaped or laterally flattened seeds with a straight awn.

LEMMA. 6.0–10.0 (mostly 7.4–8) mm. in length, exceeding palea and grain, usually laterally concave, the sides slightly upturned or nearly vertical; sometimes upturned on the lower part (rachilla end) of the seed and flaring at the apex. Dorsal surface glabrous or puberulent near margin; longitudinal nerves obscure except for midvein; cross wrinkles usually not present. Apical teeth 0.5–1.0 mm. long (Fig. 14a). Awn 5–10 mm. in length, usually straight, sometimes backwardly directed.

PALEA. Shorter than lemma by 0.2–1.5 (usually 0.3–0.9) mm., the difference between length of the lemma and palea being greatest on seeds with a long lemma; length subequal to grain in mature seeds. Keel marginal, teeth 0.4–0.5 mm. in length, spaced at intervals of 0.3–0.4 mm. (Fig. 13d).

RACHILLA. 1–1.5 mm. in length, short and thick, or slender, distinctly bowed, with the apex frequently located between or below edges of lemma, the apical scar thus not visible in side view, glabrous or pubescent; apical scar oblique (Fig. 15a).

GRAIN. Thin or somewhat thickened, nearly flat with the edges upcurved, or folded longitudinally (Fig. 17h).

DISCUSSION. This species is a common annual weed in the eastern United States. It is abundant in fields, along roadsides, and waste places. Its seeds are frequently found with those of agricultural forage grasses.

Seeds of *Bromus commutatus* are sometimes difficult to distinguish from those of *B. racemosus* and *B. secalinus*.[4] Distinctions are outlined in the analytical key. Seeds of *Bromus commutatus* and *B. racemosus* usually do not occur together in agricultural seed since *B. commutatus* is primarily an eastern weed, whereas *B. racemosus* is most abundant on the west coast. Differences between *B. secalinus* and *B. commutatus* are further discussed under the former.

### Bromus inermis Leyss. Smooth brome
(Figs. 11, 13c, 17a.)

OVER-ALL APPEARANCE. A large, thin, flat seed; awn reduced or absent.
LEMMA. 8–11 mm. long, 3 mm. wide, equaling or slightly exceeding grain and palea in length, distinctly wider than grain, flat or the lateral margins slightly upturned. Dorsal surface glabrous or sparsely puberulent; longitudinal nerves 5 in number, weak except for midvein. Apical teeth reduced, indistinct. Awn frequently absent, when present, inconspicuous,1–3 mm. long, straight. Callus glabrous.

PALEA. Nearly flat, approximating the grain in length; keels not marginal on mature seeds; keel bristles very short (less than 0.1 mm. in length, *see* Fig. 13c).

RACHILLA. 0.5–2 mm. in length, slightly enlarged upwards, puberulent or occasionally glabrous, nearly straight; apical scar oblique or nearly vertical.

GRAIN. Flat or slightly boat-shaped, fragile (*see* Fig. 17a).

DISCUSSION. *Bromus inermis* is extensively employed for forage in the north-central United States and adjacent Canada.

### Bromus japonicus Thunb. (Bromus arvensis, in part, of some author's
Japanese brome
(Figs. 1, 15b, 17g.)

OVER-ALL APPEARANCE. Fragile-appearing, boat-shaped or laterally folded seeds, commonly with a bent or divergently directed awn.

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[4] Illustration labeled *Bromus racemosus* in the standard Hillman-Henry plates (4, plate 3, No. 8) is probably *B. commutatus*.

LEMMA. 7–8 mm. long, longer and broader than grain and palea, exceeding palea and grain by 0.9–1.2 mm., usually strongly concaved or folded, sometimes flat. Surface glabrous or finely short-hairy along margins, 5–9 nerved, the lateral veins weak. Awn 5–10 mm. in length, commonly backwardly bowed. Callus glabrous.

PALEA. Approximating the grain in length, frequently nearly hidden from view by the incurved lemma and folded grain; keels marginal, the bristles not crowded, about 0.5 mm. in length.

RACHILLA. 1–1.5 mm. in length, upwardly enlarged, strongly bowed or nearly straight, usually short-hairy; apical scar vertical (Fig. 15b).

GRAIN. Mature grain thin and fragile, boat-shaped, folded or nearly flat, frequently the lower portion folded, the upper flaring out (Fig. 17g).

DISCUSSION. *Bromus japonicus* occurs in most of the United States with the exception of the southeastern portion, but is most common in the north-central states. Its seeds are one of the most common weedy contaminants of commercial smooth brome (*B. inermis*) seed. Occasionally seeds of *Bromus japonicus* occur in seed lots also containing *B. commutatus*. The seeds of these two species are superficially similar, but those of *B. japonicus* can ordinarily be distinguished by the vertical rachilla scar and longer pubescence on the rachilla; the folded shape, and frequent presence of a backwardly directed awn may also be helpful.

This species is termed *Bromus arvensis* in much of the older American botanical literature. The latter is a European species, closely related to *B. japonicus*, but of rare occurrence in the United States. The name *B. arvensis* is currently used by some American botanists who do not believe the two species to be distinct.

### Bromus mollis L. (Bromus hordeaceus authors, not L.) Soft chess
(Fig. 3.)

Seeds similar to those of *Bromus commutatus* from which they differ as follows: 1. lemma finely villous-hairy, distinctly nerved and cross-wrinkled; 2. seeds usually flat or slightly curved (but immature seeds may become longitudinally folded in drying).

Soft chess is distributed throughout much of the United States. It is most common in the Pacific coast states.

Knowles (7) has reported that this species and *B. racemosus* readily hybridize, and that the offspring are fully fertile.

### Bromus racemosus L.
(Fig. 4.)

Seeds similar to those of *Bromus commutatus* from which they differ as follows: 1. seeds usually flat (but immature ones may be longitudinally folded); 2. palea commonly with a distinct longitudinal, medial furrow; 3. lemma more plainly 5–7 nerved, frequently laterally wrinkled.

The seeds are also similar to *B. mollis,* differing primarily in that the lemma is not villous-hairy. The palea is variable in size in *B. racemosus,* although most frequently approximating the grain in length.

*Bromus racemosus* is common in the Pacific states east into the Rocky Mountains. It is occasionally adventive in the eastern United States.

### Bromus rigidus Roth. Ripgut grass
(Figs. 12, 13b, 16b.)

OVER-ALL APPEARANCE. A long, harsh seed with an extremely long, stiff awn.

LEMMA. 20–25 mm. long, strongly concave, the margins erect or incurved. Dorsal surface scabrous-pubescent, commonly purple. Apical teeth membranous, 4–5 mm. long. Awn very stiff, rough-scabrous, 30–50 mm. long. Callus pubescent with upwardly directed hairs over entire surface or margin only (Fig. 16b).

PALEA. Commonly nearly concealed within the grain to which it is equal in length. Keel bristles short, stiff, not crowded (Fig. 13b).

RACHILLA. 2–3 mm. long, slightly bowed and thickened upwards, short-pubescent.

GRAIN. Much shorter than lemma, not reaching base of apical teeth, rather thick, strongly concave, the lateral margins frequently contiguous.

DISCUSSION. This plant is a common weed in the West from British Columbia to Mexico and westward. It is especially prevalent in California. The long, sharp awns are dangerous to livestock, piercing and causing infections on the face.

### Bromus rubens L.
(Fig. 8.)

OVER-ALL APPEARANCE. A slender, flattened seed with a long awn.

LEMMA. 10–14 mm. long exclusive of awns, nearly flat except for upturned margins, sometimes backwardly curved. Dorsal surface usually villous, commonly purplish when mature. Apical teeth membranous, 3–4 mm. long. Awn 15–20 mm. long, frequently backwardly bent. Callus glabrous or marginally villous.

PALEA. Nearly flat, finely hairy, commonly 3-nerved or furrowed when mature. Keel bristles capillary, sometimes variable in length.

RACHILLA. 2–3 mm. long, commonly villous, nearly straight.

GRAIN. Nearly flat, thin.

DISCUSSION. *Bromus rubens* is common in the western United States. The seeds may cause injury to livestock.

**Bromus secalinus** L. Cheat, Chess

(Figs. 6, 17f.)

OVER-ALL APPEARANCE. Thick or slender, laterally folded seeds with a short awn.

LEMMA. 6.0–7.8 mm. long, 6.8–7.5 on most seeds, strongly concave or folded, the lateral margins frequently contiguous and involute, hiding the palea and grain from view. Dorsal surface nearly glabrous; venation obscure except for midnerve; cross wrinkles rarely present. Apical teeth 0.5–1 mm. long. Awn rudimentary or 1–6 mm. long, straight or with an abrupt twist or curve at or below the middle. Callus glabrous or laterally short-hairy.

PALEA. Usually approximating the lemma, sometimes shorter (to −0.3 mm.), or slightly exceeding it, frequently partially hidden within the grain and scarcely discernible; keel marginal or nearly so, the bristles approaching 0.5 mm. in length and spaced 0.3–0.4 mm. apart.

RACHILLA. 1–1.5 mm. in length, short and thick or rather slender, inconspicuously short-pubescent; distinctly bowed, on mature seeds commonly bent slightly outwards so that the free end is above the edge of the lemma, i.e., can be seen in lateral view; apical scar oblique.

GRAIN. Mature grain subequal to palea and lemma, 0.2–0.5 mm. thick, much heavier than that of any related species; sides vertical or inwardly curved; ventral groove very narrow (Fig. 17f).

DISCUSSION. Cheat is locally abundant throughout the United States. It is generally considered to be the worst weed pest among our naturalized bromes and is designated as noxious by the agricultural seed laws of many of our central and southern states. It is perhaps most feared in winter wheat. Its seeds are common in seed of wheat, southern grown oats, and various forage grasses.

The proper identification of seeds of cheat and related species of *Bromus* is frequently a matter of concern to seed technologists. Differences of opinion concerning the identity of such seeds have on several occasions resulted in misunderstandings between buyer and seller, or between commercial concerns and law enforcement agencies.

Seeds of *Bromus secalinus* are most easily confused with those of *B. commutatus*. These seeds frequently occur in mixtures in agricultural seed lots and precise determination of the number of each is difficult. Seed analysts generally attempt identification on the basis of shape. *B. secalinus* seeds usually appear short and stubby, or slender with the lemma margins involute; those of *B. commutatus* are, for the most part, wider, flatter, and flared out at the tip. However, immature *B. secalinus* may be strongly flared, and *B. commutatus* is not uncommonly laterally rolled and narrow. Observations have indicated that too much

reliance must not be placed on these distinctions, that the length of the lemma and relative length of the palea and lemma appear to be more dependable diagnostic characters.[5] The weight of seed, the nature of the awn (if not broken), the degree of flare at the tip of the lemma must, of course, be given due consideration but in a secondary role. No character or combination of characters is entirely reliable. These
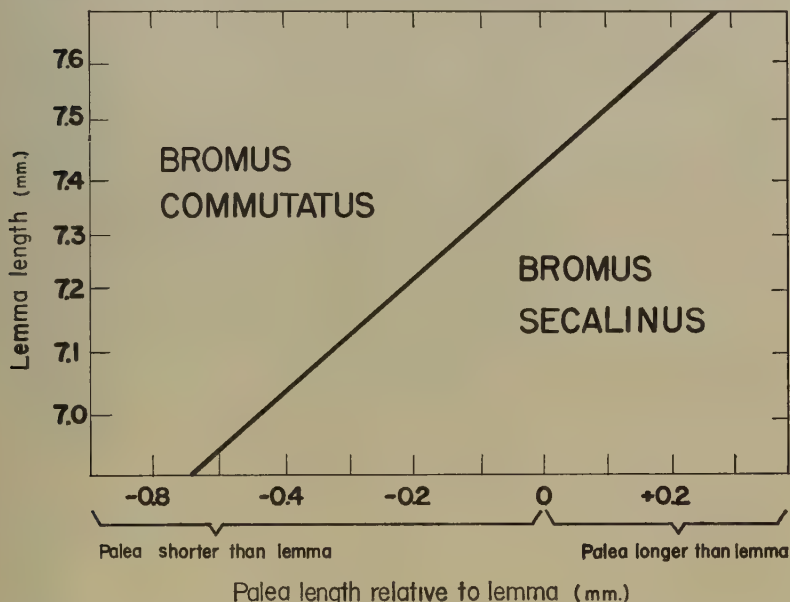


Fig. 18.—Relationship of lemma and palea length in *Bromus secalinus* and *commutatus*. If locus of lemma and palea length lies above diagonal line, seed is *Bromus commutatus;* if below, it is *B. secalinus.*

conclusions have been verified by the senior author (Isely, in press) in a special investigation of the seeds of these two species. In addition, he reported that while thick, heavy seeds are always *B. secalinus,* the thinner, lighter seeds may represent either species. He noted that, while the palea generally approximates the lemma in *B. secalinus* and is shorter than the lemma in *B. commutatus*, in both species the relationship between the palea and lemma is somewhat dependent upon the total length of the lemma. This relationship, diagrammed in Fig. 18 (adapted from the data presented in above cited paper), should be useful in interpreting the seeds of these species.

Seed analysts will continue to encounter difficulty in classifying seed

[5] Use of ocular micrometer in making such determinations is discussed under procedures.

material: 1. which consists of dehulled grains; 2. in which the tip of the lemma and palea have been destroyed; 3. in which all *Bromus* seeds are excessively small; 4. in which seeds appear intermediate between *B. secalinus* and *commutatus*. The first three conditions result from milling to which the seeds have been subjected. Dehulled grains cannot always be determined with certainty. Mature grains of *B. secalinus* are much heavier than those of *B. commutatus*, and commonly possess a slightly thickened flange or rim along the margin. Immature grains are more difficult to determine and one is frequently forced to predicate their identity on the basis of unhulled seeds occurring in the same seed lot. Seeds in which the tip of the lemma has been destroyed during the processing may be difficult to classify because the length of the lemma cannot be accurately determined nor can it be properly compared with the palea; further, loss of the awn accompanies destruction of the tip of the seed. The authors' experience has indicated that such seeds are most frequently those of *B. commutatus*. The greater length of the lemma beyond the grain (as compared to *B. secalinus*) probably renders it more liable to destruction. The presence of uniformly small seed of *B. secalinus* and *commutatus* in certain agricultural grass seed lots (usually fescues) is apparently due to the fact that cleaning procedures are successful in removing the larger seeds, but do not touch the small ones which more closely approximate the size of the crop seeds. While such seeds may be initially puzzling, since they do not conform to expected size characteristics, they can usually be distinguished as described above.

The Iowa State College Seed Laboratory has encountered several agricultural seed samples containing certain *Bromus* seed which could not be definitely associated with either *B. secalinus* or *commutatus*. Usually most of the seeds were *B. commutatus*, but contained an admixture of seeds bearing some characteristics of *B. secalinus*. Progeny from some of these seeds have been examined in greenhouse plantings. In general, plants could be readily identified if they came from seed typical of one of the two species, but progeny of aberrant seed were commonly intermediate in characters. Progeny seed was similar to that of the parent.

These observations naturally suggest that hybridization between *Bromus secalinus* and *B. commutatus* may occur (perhaps only in certain areas or under specific circumstances) and that fertile hybrids backcross with parents, particularly *B. commutatus*. However, we have no proof of such hybridization. The techniques required — field mass collections and genetical studies — to elucidate the relationships between these species are not within the scope of the present study.

If confronted with intermediate seeds of these *Bromus* species, a logical procedure on the part of the seed technologist appears to be a careful evaluation of the various differential characters of the individual seeds, and their association with the species most closely resembled. If Fig. 18 is employed as a standard, such determinations can be made in a consistent manner.

### Bromus sterilis L.
(Figs. 9, 16a, 17c.)

OVER-ALL APPEARANCE. A stiff, slender seed with a very long awn.

LEMMA. 12–18 mm. in length, exclusive of awn, laterally concave, the margins incurved and approaching one another. Dorsal surface commonly red-purple or purple-black (mature seeds), scabrous-roughened, conspicuously 5–7 nerved. Lateral margins membranous, extending upwards into conspicuous apical teeth 2–3 mm. in length. Awn scabrous, rigid, straight, 20–25 mm. long; callus with marginal tufts of hairs (Fig. 16a).

PALEA. Shorter than lemma, approximating grain, nearly concealed within ventral furrow of grain. Keel marginal, the teeth short, inconspicuous, subequal in length.

RACHILLA. Approximately 2 mm. in length, slender, straight, slightly enlarged towards apex, glabrous or finely scabrous; apical scar vertical.

GRAIN. 9–11 cm. in length, very slender, with a deep, narrow, ventral furrow (17c).

DISCUSSION. This weed occurs throughout the United States but is infrequent in the central states.

### Bromus tectorum L. Downy brome
(Figs. 10, 13a, 14b, 17e.)

OVER-ALL APPEARANCE. A backwardly bent, slender, hairy seed with a long, flexuous awn.

LEMMA. 7–11 mm. long exclusive of awn, concave, the margins vertical or slightly incurved, but not coming together. Entire lemma (and grain within) usually backwardly curved, frequently purplish in color. Dorsal surface villous or rarely glabrous; lateral margin with a narrow membranous border which is upwardly continued into a pair of very conspicuous apical teeth 2–3 mm. long (Fig. 14b). Awn stiff, straight or backwardly curved, 10–14 mm. long. Callus with a tuft of hairs on each side, otherwise glabrous.

PALEA. Approximating grain but shorter than lemma; surface finely hairy; keels marginal or submarginal, the bristles of irregular, capillary hairs various in length (Fig. 13a).

RACHILLA. 2–3 mm. long, straight, slightly enlarged upwards, villous; scar vertical.

GRAIN. Shorter than lemma, reaching to about base of apical teeth, narrowly oblong, about twice as wide as high, boat-shaped, strongly grooved (Fig. 17e),

DISCUSSION. Perhaps our commonest weedy brome, *Bromus tectorum*, is widely distributed throughout the United States.

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# CAROTENOID CONTENT OF TOMATO FRUITS AS INFLUENCED BY ENVIRONMENT AND VARIETY

## I. EFFECT OF TEMPERATURE AND LIGHT [1]

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The carotenoid pigments of fruits and vegetables have received considerable attention from research workers in recent years. This interest arises from the importance of this group of pigments in human nutrition, in determining the acceptance of fruits and vegetables and in physiological studies.

Duggar (3) was the first to show the pronounced effect of temperature upon lycopene formation in the tomato. He found that fruits picked green and ripened off the vine showed an optimum temperature of 18° to 23°C. for maximum lycopene development. Above 30°C., lycopene development was suppressed and practically no red coloring appeared in the fruits, although they did soften and turn yellow. When these yellow fruits were placed at temperatures near 20°C., red coloring appeared, indicating that the suppression of lycopene production at high temperatures did not destroy the capacity for later development. In studies of detached green fruits, Rosa (22) obtained the most rapid coloring at 25°C., Vogele (25) found 24°C. to be the optimum; and Denisen (2) found an optimum range from 20° to 25°C. for the maximum coloration of both red (Rutgers) and orange (Jubilee) tomatoes.

Haber (5) reported that fruits picked at the mature-green stage and stored at 2° to 5°C. fail to ripen properly in storage. MacGillivray (15), Duggar (3), and Went, LeRosen, and Zechmeister (26) have shown that fruits ripened on the vine have a temperature response similar to those picked green and ripened in storage.

It has been shown by Duggar (3), Howard (7), Rosa (22), Vogele (25), Went, LeRosen and Zechmeister (26), and Denisen (2) that light is not essential for lycopene production for fruit picked green and ripened in storage. Smith (23) was the first to show that tomato fruits produce lycopene when grown in complete darkness from fruit set to maturity.

Smith (23) also made a study of the effect of light wave length on carotenoid formation in tomato friuts. Violet rays gave the largest pro-

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duction of carotenoids and yellow light the smallest. His data indicate that wave lengths between 5400 and 5800 A. are not conducive to the production of carotenoids. He also found that protection from intense light favored lycopene formation, but that fruits receiving more light showed a greater development of carotene.

MacGillivray (15) studied the interaction of temperature and light on carotenoid development. He found that the sides of fruits facing the sun were higher in temperature than the shaded sides; and fruits protected by foliage were from 13° to 25° cooler than those exposed to the sun. Fruits that had the poorest foliage protection showed the greatest daily range in temperatures and had the poorest color. A previous investigation by the present author (2) has shown that shaded fruits are superior in color to those exposed to full sunlight.

Although very little information is available on the influence of plant nutrients on the carotenoid content of tomato fruits, various workers have investigated the relationships of nutrients to the carotene content of other plant materials. Bernstein, Hamner, and Parks (1) found no appreciable effect of macronutrients on the carotene content of turnip greens. Ijdo (8) found that very large amounts of nitrogen resulted in an increased carotene content in spinach leaves. Moon (19) obtained significant increases in carotene content of pasture grasses from the application of both nitrogen and potassium, with the greatest increase coming from nitrogen.

Duggar (3) found that increasing the oxygen content of the atmosphere hastened the reddening of tomatoes at room temperature. Lycopene was not produced when green fruits were ripened in nitrogen, hydrogen, or carbon dioxide. Vogele (25) also found that oxygen is necessary for lycopene production. Furlong (4) found that an atmosphere consisting of 5 per cent oxygen, 10 per cent carbon dioxide and 85 per cent nitrogen delayed the ripening of tomato fruits by nine days (over that in air).

Rosa (22) found that a concentration of ethylene at or lower than 1:4300 accelerated the development of red color in mature-green tomatoes. Work (27) reported that ripening is hastened by a three- to four-day treatment with ethylene. From a commercial standpoint, Raleigh (21) states that some markets discriminate against ethylene-ripened fruit. House, Nelson, and Haber (6) showed that ripe fruits were a richer source of vitamin A than green fruits, but found no significant differences in the vitamin A content of green, air-ripened, ethylene-ripened, or vine-ripened fruits.

The inheritence of skin and flesh color in yellow and red tomatoes has been established by Lindstrom (12). The dominant gene (R) causes red flesh and the recessive gene (r) gives yellow flesh color, whereas the independent, dominant Y produces yellow skin and y results in colorless skin. Since these two sets of genes are carried on separate chromosomes, four genotypes can result in the gametes. MacArthur (14) discovered another pair of genes controlling flesh color in ripe fruit. These are the T and t and are called "tangerine." A brilliant orange

(tangerine) color results when the double recessive *tt* is present in the genetic makeup.

The relation between the genotype and the carotenoids of the tomato was established by LeRosen, Went, and Zechmeister (10). They found that the gene pairs *Rr* and *Yy* which are not related genetically are also unrelated in their chemical effect. The *R* gene influences the development of the red and yellow plastid pigments. The *Y* gene has no control over the plastid pigments, but when present it causes about a ten-fold increase in an alkali-soluble, unidentified yellow pigment in the epidermis of the fruit.

Zechmeister, *et al* (28) discovered the carotenoid pigment, pro-lycopene, in the Tangerine tomato. This carotenoid, a stereoisomer of lycopene, is the compound formed when the double recessive *tt* is present in the genotype of a tomato.

Howard (7) found varietal differences in the lycopene content of four red-fruited tomato varieties. MacGillivray (16) reported very small differences among six varieties of red tomatoes when the external color of ripe fruits was measured. Lincoln, *et al* (11) found that the common varieties of red tomatoes, *Lycopersicum esculentum*, contained from 32 to 111 micrograms of lycopene per gram of fresh fruit and the wild cur-rant type, *L. pimpinellifolium*, ranged from 37 to 463. Porter and Zscheile (20) reported the presence of prolycopene in the Tangerine and Jubilee varieties to the extent of about 40 micrograms per gram of fresh fruit.

The present author (2) found that both shaded and exposed fruits of the varieties Rutgers, Marglobe, and U. S. No. 24 were superior in color to Indiana Baltimore, Pritchard, and Pan America.

## MATERIALS AND METHODS

### TEMPERATURE EFFECTS

Although numerous workers have shown the pronounced effect of temperature in the development of red color in tomatoes, it was felt that further information could be gained by varying the temperature in a way to simulate the normal day and night cycle. Mature-green fruits of the Rutgers variety were picked, graded, and placed in incubators at various temperatures for ripening. The fruits were placed in ventilated cellophane bags and were transferred from one incubator to the other at twelve-hour intervals. Four sets of temperature fluctuations were used: 35° and 15°, 30° and 20°, 15° and 25°, and 25° and 35°C. A ran-domized block design was used and consisted of three fruits per bag, one bag per treatment, four treatments, and four replications of the experiment.

### LIGHT EFFECTS

In studying the effects of light on the development of carotenoid pigments in the tomato, two types of experiments were conducted. One concerned the influence of wave length of light, and the other compared

the weekly development of pigments under conditions of normal amounts versus no light.

Subjecting the developing fruits to various light wave lengths was accomplished by placing colored cellophane bags of known transmission around the fruit at the time of fruit set. The bags were placed on tomato clusters having three or more fruits or blossoms which were shaded by plant foliage. Usually one or two of the blossoms of a cluster had set fruit at the time of bagging; the remainder were hand-pollinated before bagging.

In addition to the use of colored cellophane, other treatments were also used. The check consisted of using clear transparent cellophane bags on the fruit to reduce differences due to temperature and humidity. An unbagged group was also included so as to compare color development inside and outside of bags. Light was completely excluded in one group in which bags of aluminum foil were placed around the fruit clusters.

This experiment was conducted under both greenhouse and field conditions (with some slight variations). The Rutgers variety of tomatoes was used in both series. In the greenhouse the treatments were located at random on sixteen tomato plants divided into four replications. In the field (where the plants had more spreading growth and larger numbers of blossom clusters) each of four plants contained all the treatments of one replication. In both the field and greenhouse trials, each treatment consisted of at least three fruits, of which three were used for the pigment analysis.

For the greenhouse studies, violet and red cellophane bags were dyed in the laboratory and used for bagging fruit. This was accomplished by dipping plain cellophane bags for one minute in saturated solutions of acid fuchsin and crystal violet respectively. Other treatments used in the greenhouse consisted of using bags of tango (commercial orange colored cellophane), tango plus red, plain, and aluminum foil. Clusters were also left unbagged as checks. The tango bags were made of double thickness cellophane and the red plus tango bags were made up of a single thickness of each of the two colors. Spectral transmission readings on all of the materials used were obtained by placing a layer of cellophane on one side of a cuvette in a Coleman Universal Spectrophotometer. The data are shown in Fig. 1.

The cellophane bags used in the greenhouse were replaced periodically during the experiment (when they started to fade). In the field trials it was necessary to change the bags at one- to two-week intervals and in some cases oftener because of fading and weathering.

In both the greenhouse and field trials, temperatures were taken both inside and outside of the bags. There was an average increase in temperature of 2°C. inside the bags in the greenhouse, and 5° to 6° for those in the field. There were no consistent temperature differences among the bags of various colors. Temperatures inside the aluminum foil bags were the same as those outside. The problem of water vapor condensing inside the cellophane bags was minimized by punching a

few small holes in the bags. Although the humidity was lowered by this technique, the temperature was not appreciably affected.

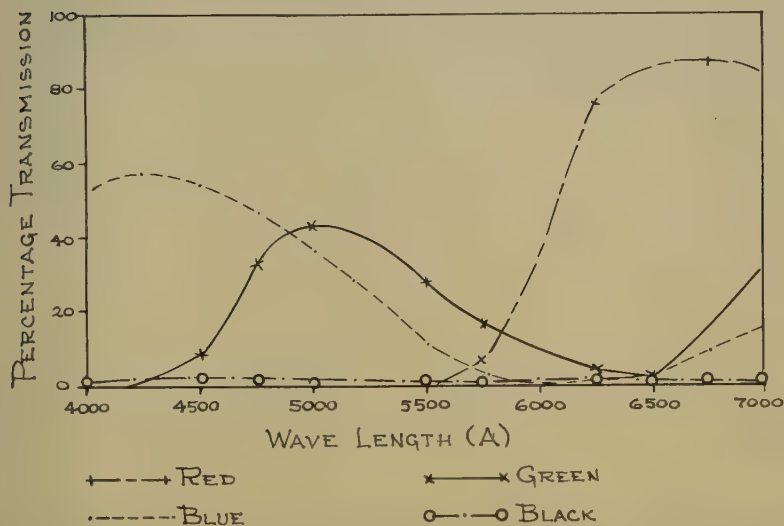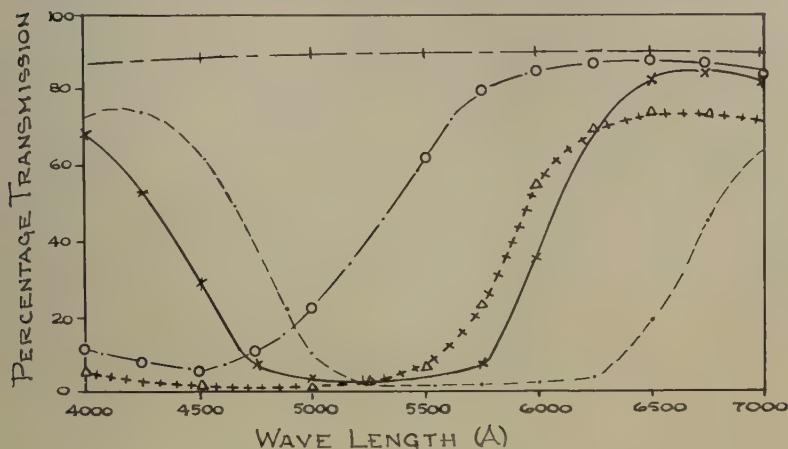In the experiment comparing the effects of light vs. no light, alum-



FIG. 1.—Transmission curves of the nine types of cellophane used in the experiment on light.

inum foil bags were used as the "dark" treatment and normal foliage
cover as the "light" treatment. Blossom and fruit clusters were selected
and pollinated as before. One cluster of each of the bagged and unbagged
fruits of each of four replications was a separate treatment of the time
element. Clusters were analyzed at three-, four-, and five-week periods
after fruit set. The Rutgers variety was used in this experiment.

<div align="center">MEASUREMENT OF CAROTENOIDS</div>

Pure lycopene crystals were obtained by chromatography using a
column of a 1:1 mixture of activated magnesium oxide and Celite (a
commercial diatomaceous earth) (Strain, 24). The lycopene band was
removed from the column and eluted with a 1:1 mixture of benzene
and ethyl alcohol and weighed. Concentrations of 1.00, 0.75, 0.50, and 0.25
parts per million in petroleum ether (Skelly B) were prepared for
spectrophotometric readings. Ten milligrams of commercial 99.5 per
cent pure β-carotene crystals were dissolved in Skelly B and prepared
in concentrations of 2.00, 1.50, 1.00, and 0.50 parts per million.

Transmission readings of the various concentrations of lycopene
and carotene were obtained using a Coleman Universal spectrophoto-
meter. Typical lycopene and carotene curves were obtained which showed
the low point on the lycopene curve at 4700 A. and the low point on the
carotene curve at 4500 A.

Specific absorption coefficients were determined for each concen-
tration of lycopene and carotene by applying Beer's law as described
by Miller (17). Mean specific absorption coefficients were 300.9 for
lycopene and 155.6 for carotene.

In establishing a method of pigment analysis by spectrophotometric
means, an attempt was made to measure both the carotene and lycopene
present in the same solution [Miller (18) and Zscheile and Porter (29)].
In five known ratios highly erroneous estimates of the two carotenoids
resulted. It was found that as the proportion of lycopene to carotene
approached that of tomato extracts, lycopene exerted a tremendous
masking effect on carotene. This influence of the more highly colored
lycopene pigment was so pronounced that the specific absorption coeffi-
cient curves for tomato extracts containing lycopene and carotene very
nearly followed the curve for pure lycopene. (Fig. 2.) Presumably
Zscheile and Porter (29) were more successful in accomplishing quanti-
tative measurements of the two carotenoids in the same solution because
of the very much greater sensitivity of their spectrophotometer.

Since the transmission curves for the extracts containing the com-
bined pigments so closely approached that of pure lycopene, the specific
absorption coefficient for lycopene was used in converting the trans-
mission readings to micrograms per gram and the results were expressed
as total lycopene.

Although it is possible to separate carotene and lycopene by chro-
matographic adsorption, it was not feasible to use this time-consuming
procedure for the large numbers of samples to be measured. The xantho-

phyll pigments, however, were separated from the other carotenoids by alcoholic extraction, and spectrophotometric determinations of these pigments were made. The results obtained from the preliminary work of standardizing the spectrophotometer for carotene analysis were used as the standard for xanthophyll because of the similar colorimetric behavior of these two yellow carotenoid pigments. The conversion of transmission readings to micrograms of carotenoid per gram of fresh fruit was accomplished with the formula devised by Zscheile and Porter (29).
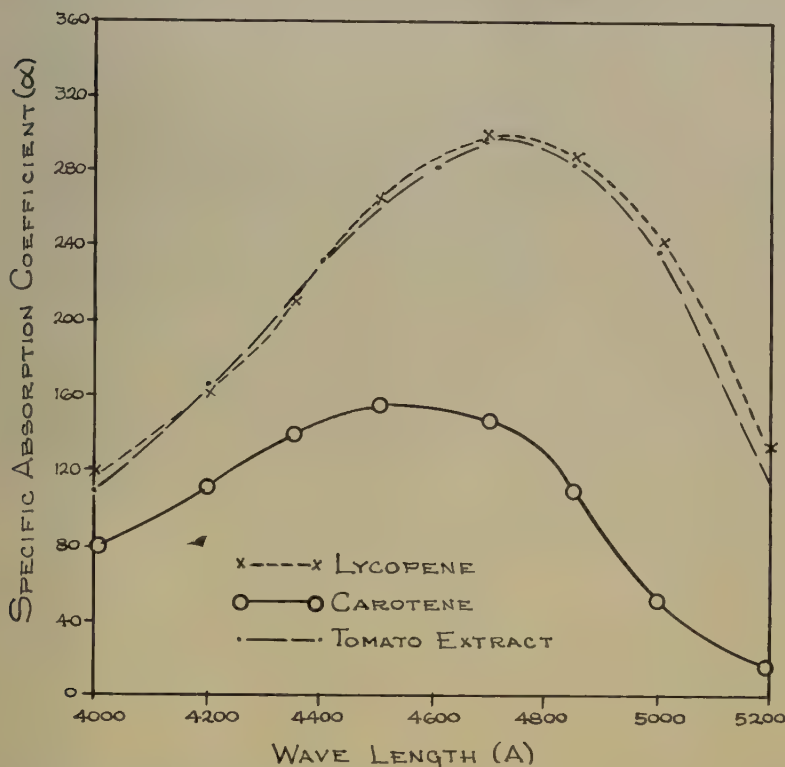


FIG. 2.—Specific absorption coefficients of lycopene, beta-carotene, and tomato pigments in petroleum ether.

A slight modification of the extraction method of Zscheile and Porter (29) was used in obtaining the tomato pigment extracts. Skelly B (principally hexane) was used instead of pure hexane. The chlorophylls were saponified before removal of the xanthophyll so the latter would not be contaminated. In some cases the resulting solution of carotenoids was not entirely clear because of the presence of water in

the Skelly B. When this occurred the flasks were placed in a desiccator containing calcium chloride until clear.

The extraction method outlined by Kramer and Smith (9) was used in the analysis of total pigments of the tomatoes produced in the experiment with plant nutrients. However, acetone was substituted for benzene, since it was found that a considerable amount of pigment was lost when the latter was used.

The chlorophyll determinations on immature green fruits were made on a du Boscq colorimeter using Guthrie's standard (13). The chlorophyll was saponified and extracted according to the method described by Loomis and Shull (13).

### SAMPLING METHODS

Ripe fruits harvested for pigment analysis were selected on the basis of ripeness, shading by foliage and size. An effort was made to develop a standard of maturity so that all fruits analyzed would be of the same degree of ripeness. The relative firmness of the fruit, the ease with which they could be detached from the vine and the complete disappearance of green color at the calyx end were measures of maturity which were used in judging ripeness. The tomatoes selected were taken from locations on the plant where they received at least partial shade during the greater part of the day. Size was taken into consideration only insofar as was typical for the varieties.

Three fruits of each treatment per replication were used for analysis in most of the experiments. In the phase of the investigation dealing with plant nutrients each fruit of the three-fruit samples was analyzed separately. In other phases a homogenized composite sample of the three fruits was analyzed.

### RESULTS AND DISCUSSION
#### INFLUENCE OF TEMPERATURE

The time required for ripening of the mature-green fruits at cycled temperatures ranged from thirteen to twenty days as shown below:

| Temperature Cycle | | Ripening Time (days) |
|---|---|---|
| 12 hrs. at: | 12 hrs. at: | |
| 15° | 35° C. | 20 |
| 20° | 30° C. | 13 |
| 15° | 25° C. | 18 |
| 25° | 35° C. | 16 |

Lycopene and xanthophyll production at the four sets of temperatures is shown in Table 1. Highly significant differences were obtained for both pigments. The means for the two temperatures of each treatment are included in Table 1 to point out the importance of the mean

daily temperature in pigment development of ripening tomato fruits. It is noted that the highest yield of lycopene was found when the temperature cycle of 15° and 25°C. was used (mean 20°). All investigators who have studied the effect of temperature on lycopene development have found the optimum to be close to 20°C. consequently, this temperature was used as a standard for comparison of the other treatments. When the $F$ test was applied to the treatment means, all treatments were found to be highly significant in their deviation from this standard (15° to 25°C.). It is also interesting to note that the two treatments which have the same mean of 25° do not differ greatly in their production of lycopene.

When these fluctuating temperatures are likened to day and night temperatures in the field, some interesting implications may be derived. Although the environment of the fruits was changed considerably from the field to the incubators, the results still offer a plausible explanation for better colored fruits under certain conditions. In general the tomato canning industry has found that tomato fruits grown in the field in

TABLE 1
LYCOPENE AND XANTHOPHYLL CONTENT OF TOMATO FRUITS UNDER CONDITIONS OF
FLUCTUATING TEMPERATURES

| Temperature Cycle | | | Mean Temperature | Pigment Content* | |
|---|---|---|---|---|---|
| 12 hrs. at: | and | 12 hrs. at: | | Lycopene | Xanthophyll |
| 15° | | 35° C. | 25° | 33.7 | 1.75 |
| 20° | | 30° C. | 25° | 34.7 | 2.25 |
| 15° | | 25° C. | 20° | 50.9 | 1.62 |
| 25° | | 35° C. | 30° | 17.4 | 1.79 |

\* Expressed in micrograms per gram of fresh fruit.
  For lycopene at less than 0.01, P = 11.0
  For xanthophyll at less than 0.01, P = 0.29

upstate New York and on the muckland areas of Indiana are consistently more red in color than those grown in other areas. It seems logical to assume that this superior color is due to the characteristic pattern of day and night temperatures of these areas. That is, the moderately warm daytime temperatures and relatively cool night temperatures (roughly corresponding to the 15° and 25°C. cycle) would produce fruit of higher lycopene content than would be produced under conditions of high daytime and moderately warm night temperatures as encountered during the growing season in the Midwest. This speculation is also in accord with the findings of MacGillivray (15) in which he reported that fruits which ripen late in the season when temperatures are cooler are more highly colored than the earlier maturing fruits of the same variety.

The 20°–30°C. cycle resulted in the greatest xanthophyll content. This temperature fluctuation was selected as the check since numerous

investigators have found a considerable amount of yellow color produced at temperatures above the optimum for lycopene development. The mean temperatures do not show a direct relationship to xanthophyll production as they did with lycopene. On closer scrutiny one may observe a decreased development of xanthophyll in each case where 15° or 35°C. was used. Apparently these temperatures represent unfavorably low and/or high conditions for xanthophyll production. This observation offers a possible explanation for the poorer coloring of artificially ripened fruits such as appear in the grocery stores during the winter months. As a rule these fruits are pinker than vine ripened fruit of the same variety. The brilliant red of vine ripened fruits is due not only to the high lycopene content, but also to the blending of the yellow pigments with the red. Fruit picked at the mature-green stage for commercial ripening in storage is held at temperatures low enough to prevent deterioration of the fruit yet sufficiently high for color development. Consideration of these facts suggests that a temperature range of approximately 15° to 20°C. would be suitable. Based on the data obtained this would result in decreased development of the xanthophyll pigments and lack of the warm brilliant red of ripened fruits even though the lycopene may show considerable development.

The appearance of the fruit from the 20°–30°C. cycle treatment was not attractive because of a mottled red and orange surface when ripe. The poorest colored fruits were produced by the 25°–35°C. cycle; only a red blush developed over a yellow background.

### INFLUENCE OF LIGHT

Lycopene content of tomato fruits grown and ripened under the various light treatments in the greenhouse is shown in Table 2. Significant differences were found among treatments. Apparently, exclusion of light (by aluminum foil treatment) interferred seriously with the production of lycopene. It is interesting to note that in no case did fruit grown in colored cellophane bags produce quantities of lycopene sig-

TABLE 2

LYCOPENE CONTENT OF TOMATO FRUITS
RIPENED ON THE VINE IN THE GREENHOUSE
AS INFLUENCED BY BAGGING

| Bagging Treatments | Lycopene Content* |
|---|---|
| Red and tango cellophane.......... | 75.0 |
| Red cellophane........ | 79.2 |
| Violet cellophane...... | 85.8 |
| Tango cellophane...... | 73.8 |
| Clear cellophane....... | 81.5 |
| Aluminum foil......... | 36.3 |
| Unbagged............ | 63.7 |

\* Expressed in micrograms per gram of fresh fruit.
At less than 0.05, $P = 14.9$

niticantly different from those in plain cellophane. However, all treatments involving cellophane bags resulted in increased production of lycopene over the unbagged fruit.

The results for the field experiment are shown in Table 3. The tango, black, and aluminum foil treatments gave significantly lower lycopene contents in the fruit than the clear cellophane. Although the unbagged fruits yielded less lycopene than the check, the difference was not significant. Perhaps the cooling effect of the wind lowered the temperature of the unbagged fruits in the field and resulted in greater lycopene production than was obtained indoors. Also as was pointed out earlier the increase in temperature of bagged over unbagged fruits in the field was greater than in the greenhouse. Consequently it is to be expected that the lycopene production would be lower in bagged fruits in the field than in those in the greenhouse.

TABLE 3

LYCOPENE AND XANTHPOHYLL CONTENT OF FIELD GROWN TOMATO FRUITS AS INFLUENCED BY BAGGING

| Bagging Treatment | Pigment Content* | |
| --- | --- | --- |
| | Lycopene | Xanthophyll |
| Green cellophane | 67.2 | 1.75 |
| Red cellophane | 74.6 | 1.70 |
| Violet cellophane | 78.7 | 2.07 |
| Tango cellophane | 62.2 | 1.62 |
| Blue cellophane | 67.7 | 1.71 |
| Black cellophane | 57.0 | 1.75 |
| Clear cellophane | 75.9 | 1.63 |
| Aluminum foil | 40.1 | 0.98 |
| Unbagged | 70.3 | 1.71 |

* Expressed in micrograms per gram of fresh weight.
  For lycopene at less than 0.05, $P = 9.5$; for xanthophyll at less than 0.05, $P = 0.37$.

Significant differences in xanthophyll content were obtained for the several treatments, with the aluminum foil giving significantly lower values (at the 1 per cent level) and the violet cellophane significantly higher (at the 5 per cent level) than clear cellophane. These results indicate that some light is essential for maximum xanthophyll development. Wide differences were found between the aluminum foil and black cellophane treatments. Apparently the small amount of light which passes through the black cellophane was sufficient for normal production of xanthophyll. The high content of xanthophyll in fruits grown under violet cellophane may be attributable to the short wave lengths transmitted (Fig. 1).

The fruits grown under light and dark treatments were analyzed for carotene and xanthophyll content at weekly intervals. At three weeks after fruit set some of the fruits were analyzed and others at four weeks and five weeks after fruit set. The carotene content for

these immature fruits and the analysis of variance are shown in Table 4.

Highly significant differences were obtained for treatments and significant differences were found for the interaction of weeks $\times$ treatments. The absence of light caused a very marked reduction in the carotene content. An interesting trend of carotene development with age was noted in each of the two treatments. The carotene content of the fruits decreased with age when kept in the dark, whereas it

TABLE 4

CAROTENE CONTENT OF IMMATURE FRUITS GROWN UNDER LIGHT AND IN THE DARK

| Treatment Period (Weeks after fruit set) | Carotene Content* | | |
|---|---|---|---|
| | In Dark | In Light | Mean |
| 3................... | 0.46 | 1.42 | 0.94 |
| 4................... | 0.41 | 1.71 | 1.06 |
| 5................... | 0.37 | 2.09 | 1.23 |
| Mean............... | 0.41 | 1.74 | ................... |

Analysis of Variance

| Source of Variation | df | Mean Square |
|---|---|---|
| Replications...................... | 3 | 0.0065 |
| Treatment....................... | 1 | 10.3397† |
| Error (a)....................... | 3 | 0.0782 |
| Weeks.......................... | 2 | 0.1708 |
| Weeks x treatments.............. | 2 | 0.3868‡ |
| Error (b)....................... | 12 | 0.0855 |

* Expressed in micrograms per gram of fresh weight.
† Significant at P less than 0.01.
‡ Significant at P less than 0.05.

increased with age in the light. These two trends tend to offset each other and as a result no significant differences were found for weeks.

The means for xanthophyll content of the same fruits and the analysis of variance are shown in Table 5.

Highly significant differences were found between treatments due to the greatly decreased production of xanthophyll in the fruits held in the dark. Here as with carotene, a decreasing xanthophyll content with age was found where fruits were grown in the dark. However, there was no trend toward increased production of this pigment for the fruits grown in the light. No significant differences were found for weeks or weeks $\times$ treatments. Light was found to be essential for maximum development of xanthophyll as was previously shown for fruits grown in aluminum foil bags.

A comparison can be made of the relative amounts of carotene and xanthophyll in immature fruits from the results of this experiment. In

the light treatments, fruits contained more carotene per gram of fresh weight, whereas, in the dark xanthophyll was present in relatively larger quantities, although the production of both was greatly reduced.

The immature fruits grown inside the aluminum foil bags contained no visible traces of chlorophyll. The chlorophyll content of the immature fruits grown under normal light conditions is shown in Fig. 3.

Significant differences at the 0.01 level were found due to age of

TABLE 5

XANTHOPHYLL CONTENT OF IMMATURE FRUITS GROWN UNDER LIGHT AND IN THE DARK

| Treatment Period (Weeks after fruit set) | Xanthophyll Content* | | |
|---|---|---|---|
| | In Dark | In Light | Mean |
| 3................... | 0.68 | 1.26 | 0.97 |
| 4................... | 0.51 | 1.38 | 0.94 |
| 5................... | 0.36 | 1.27 | 0.82 |
| Mean............. | 0.51 | 1.27 | ................... |

Analysis of Variance

| Source of Variation | df | Mean Square |
|---|---|---|
| Replications....................... | 3 | 0.0030 |
| Treatments........................ | 1 | 3.7525† |
| Error (a).......................... | 3 | 0.0301 |
| Weeks............................ | 2 | 0.0538 |
| Weeks x treatments................ | 2 | 0.0676 |
| Error (b)......................... | 12 | 0.2190 |

* Expressed in micrograms per gram of fresh weight.
† Significant at P less than 0.01.

the immature fruit. Decreasing chlorophyll content with increasing age of the fruit is apparent and shows very little deviation from regression values.

## SUMMARY

1. Tomato fruits were subjected to various environmental factors to determine the influence of these factors on carotenoid content.

2. Lycopene was shown to exert a tremendous masking effect over the other carotenoid pigments present in the tomato, making it possible to obtain sufficiently precise determinations of lycopene content with transmission readings of the tomato pigment extracts.

3. Mature-green Rutgers tomato fruits ripened in storage incubators at four sets of temperature cycles, ripened most rapidly when alternated between 20° and 30°C. Tomato fruits ripened at the 15° and 25°C. cycle produced the largest amount of lycopene, and the poorest colored fruits resulted from the 25°–35°C. cycling. The mean of the two

temperatures of each treatment seemed to be the determining influence for lycopene production.

4. Carotenoid development as affected by spectral composition of light was studied. In the field experiments, tomatoes grown and ripened inside orange cellophane bags were significantly lower in lycopene content than those encased in colorless cellophane bags. Violet cellophane increased xanthophyll production.

5. Lycopene and xanthophyll developed in Rutgers tomatoes that
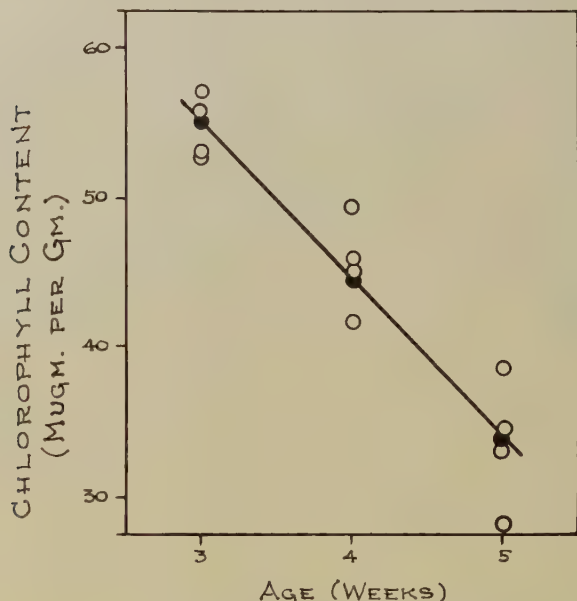


FIG. 3.—Effect of age on chlorophyll content of fresh immature tomato fruits.

were grown and ripened in the dark; however, light was essential for maximum development of both pigments.

6. The carotene and xanthophyll content of immature tomato fruits grown in the dark decreased with age, whereas an increase for carotene but not for xanthophyll was found for fruit grown in the light.

7. Chlorophyll measurements taken from fruits grown under normal light showed a uniform quantity per fruit but a decreasing percentage as the fruits increased in age from three to five weeks after fruit set.

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# CAROTENOID CONTENT OF TOMATO FRUITS AS INFLUENCED BY ENVIRONMENT AND VARIETY. II. EFFECTS OF PLANT NUTRIENTS, GAS STORAGE, AND VARIETY[1]

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This is the second and concluding report of an investigation of the carotenoids of tomato fruits. The literature review and carotenoid extraction methods were presented in Part I.

## MATERIALS AND METHODS
### PLANT NUTRIENT EFFECTS

An experiment was designed to determine the effects of low and high levels of nitrogen, phosphorus, and potassium on the carotenoid content of tomato friuits. The study was conducted in the greenhouse to permit careful controlling of nutrients and other environmental factors.

The tomatoes were grown in greenhouse boxes of dimensions 18 x 22 x 7 inches which were filled to a depth of 6 inches with Clarion fine sandy loam soil. The soil was taken from an old orchard site and was considerably depleted in nutrients. Tests showed that it was very low in available nitrogen, phosphorus, and potassium. The $pH$ was 5.35.

A factorial design involving high and low levels of the three nutrients was used. None of the specific macronutrients was supplied to the various treatments which were at low levels of the element or elements concerned. A commercial mixture of essential minor elements was applied to all treatments to avoid effects due to the deficiencies of micronutrients. Quantities of the fertilizers applied to the soil for the various treatments are shown below:

| Treatment | $NH_4 NO_3$ | Fertilizer Treatment* Superphosphate (0–20–0) | KCL |
|---|---|---|---|
| (l) . . . . . . . . . | . . . . . . . . . . . . . . | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | . . . . . . . . . . . . . . . . . . |
| n . . . . . . . . . | 8.19 | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | . . . . . . . . . . . . . . . . . . |
| p . . . . . . . . . | . . . . . . . . . . . . . . | 32.70 | . . . . . . . . . . . . . . . . . . |
| np . . . . . . . . | 8.19 | 32.70 | . . . . . . . . . . . . . . . . . . |
| k . . . . . . . . . | . . . . . . . . . . . . . . | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | 5.47 |
| nk . . . . . . . . | 8.19 | . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | 5.47 |
| pk . . . . . . . . | . . . . . . . . . . . . . . | 32.70 | 5.47 |
| npk . . . . . . . | 8.19 | 32.70 | 5.47 |

* Expressed in grams for 18″ x 22″ x 6″ of soil.

Rutgers tomato seed was planted in late August, and two weeks after sowing the seedlings were transplanted to 3-inch pots in fertile soil. After four weeks growth the healthy and vigorous plants were transplanted to the boxes containing the nutrient treatments. One plant was placed in each box and the boxes were so spaced that the plants were in two rows, 30 inches apart with 24-inch spacings within the rows. Guard plants were placed on the greenhouse bench at each end of the two rows. Four replications of the eight randomized treatments made a total of thirty-two plants in the experiment.

After the transplanted tomato plants were well established the amount of water applied was cut down to encourage the development of extensive root systems and to avoid leaching of the nutrients. The plants were trained and pruned to a single stem.

At six weeks after the final transplanting, symptoms of deficiencies became apparent at the low nutrient levels. Characteristic symptoms which were observed included purpling of the leaves and stems due to a low phosphorus condition, brown dried patches on the leaves and very small fruit due to low potassium levels and considerable yellowing of the leaves on plants grown in soil low in nitrogen. This seemed proof that the desired conditions for the several treatments had been attained. In spite of the slow growth and impaired efficiency of many of the plants, all were brought to fruiting and produced sufficient fruits for carotenoid analysis.

### GAS STORAGE EFFECTS

In designing the investigation to show the effects of gas storage on carotenoid development during ripening, detached mature-green fruits were placed in incubators at two temperatures. A split-plot design with four replications was used for this experiment. The $F_1$ fruits of a Rutgers x *Lycopersicum pimpinellifolium* cross were used.

The respiration chamber was of the type described by Loomis and Shull (2); a glass to mercury seal was used. A 1000 ml. Erlenmeyer flask was used as the storage chamber. Twelve of the small fruits were placed in each chamber.

Air, oxygen, and nitrogen were used; the fruits were stored at two temperatures (20° and 35°C.). The twelve fruits in each flask constituted one replication for each of the temperature-gas treatments. The source of oxygen was a commercial tank gas; nitrogen was obtained from air by the use of alkaline pyrogallol.

A ¼-inch layer of gravel was placed on the bottom of each respiration flask; the green tomatoes were placed on the gravel. For the air treatment the flasks were closed and the valves and stoppers sealed with mercury. An atmosphere of 60 per cent oxygen was used for the oxygen treatment, so in this case the flasks were half filled with water; the water was then displaced with oxygen. For the nitrogen treatment the gas was transferred to the flasks by displacement of water.

After the gassing treatment the per cent oxygen in each flask was determined with a Haldane gas analyzer. The results indicated that the air treatment contained approximately 20 per cent oxygen, the oxygen treatment 61 per cent oxygen and the nitrogen treatment 1.5 per cent oxygen. Four flasks of each treatment were placed in the dark in a 20°C. incubator and the same number placed in the dark in a 35°C. incubator. At the end of ten days the oxygen content of the air and oxygen treatments were readjusted.

### GENETIC EFFECTS

Several tomato varieties and hybrids were grown and the fruits analyzed to determine varietal differences in carotenoid content and to further investigate the ability of some of the varieties to transmit carotenoid-forming characters to hybrid progeny. In one phase of the experiment twelve red varieties and hybrids were used. Rutgers was chosen as the standard of comparison since it is generally accepted by the canning industry as having the best internal color. The other varieties used were Marglobe, Pritchard, Earliana, Indiana-Baltimore, Pan America, U. S. No. 24, and a wild form of *Lycopersicum esculentum*, No. 640. (The latter was obtained from the Utah Agricultural Experiment Station. It has a deep red color and was included in the experiment because it was felt that it might be a good source of breeding material.) The hybrids used were $F_1$ progeny of the following crosses: Pritchard x Earliana, Rutgers x Marglobe, Rutgers x Jubilee, and Indiana-Baltimore x Pan America.

The carotene contents of Mingold, a yellow-fruited variety, and Jubilee, an orange-fruited variety, were determined. Rutgers and Accession 160, types of *L. esculentum* and *L. pimpinellifolium* respectively, and their interspecific cross were also compared.

In all phases of this experiment the plants were arranged in the field as randomized blocks with four replications. There were five plants per replication of each species, variety and hybrid to insure sufficient numbers of ripe fruit for pigment extraction.

### RESULTS AND DISCUSSION
#### INFLUENCE OF PLANT NUTRITION

The tomato fruits grown with high and low levels of nitrogen, phosphorus, and potassium in the greenhouse were analyzed individually for lycopene content. The mean lycopene content of the fruits within each treatment and the analysis of variance (Table 1) indicate significant differences due to nutrient treatments.

Although the sampling error was high it did not differ significantly from the experimental error. This phase of the experiment was first to be conducted and because of the high sampling error per cent and wide variation of treatment means, the individual fruits were combined in

composite samples in later pigment extractions to reduce the sampling error.

Yates' (4) method for determining effects of treatments in a factorial experiment was used in separating the effects of the various nutrients. A comparison of results shown in Table 2 indicates that nitrogen was the only element which gave significant increases in lycopene content. For example the N effect shows an increase over no treatment, NP an increase over P, NK an increase over K, and NPK an

TABLE 1

LYCOPENE CONTENT OF TOMATO FRUITS AS AFFECTED BY NITROGEN, PHOSPHORUS, AND POTASSIUM FERTILIZATION

| Treatment Code | Lycopene Content† | Effects of | Mean Effects |
|---|---|---|---|
| (1). . . . . . . . . . . . . . | 59.1 | . . . . . . . . . . . . . . . . . | . . . . . . . . . . . . . . . . . |
| n. . . . . . . . . . . . . . . | 62.2 | N | 25.2* |
| p. . . . . . . . . . . . . . . | 58.9 | P | 18.6 |
| np. . . . . . . . . . . . . . | 66.0 | NP | 14.9 |
| k. . . . . . . . . . . . . . . | 53.1 | K | −14.5 |
| nk. . . . . . . . . . . . . . | 55.2 | NK | 4.7 |
| pk. . . . . . . . . . . . . . | 55.2 | PK | 11.3 |
| npk. . . . . . . . . . . . . | 68.1 | NPK | 6.7 |

† Expressed in micrograms per gram of fresh weight.
* Significant at P less than 0.05.

ANALYSIS OF VARIANCE

| Source of Variation | d.f. | Mean Square |
|---|---|---|
| Replications. . . . . . . . . . . . . . . . . . . . . . . . . . | 3 | 174.0 |
| Treatments. . . . . . . . . . . . . . . . . . . . . . . . . . | 7 | 343.8* |
| Experimental Error. . . . . . . . . . . . . . . . . . . | 21 | 135.4 |
| Sampling Error. . . . . . . . . . . . . . . . . . . . . . | 64 | 108.3 |

* Significant at P less than 0.05.
† Sampling error per cent = 17.4.

increase over PK. The effect of the phosphorus treatment approached significance; analysis of the data showed that in all cases but one the addition of phosphorus gave an increased production of lycopene. In no treatment, except the one involving a complete fertilizer, did the addition of potassium increase the lycopene content of the fruit.

It is interesting to note that the greatest production of lycopene occurred with the addition of NPK, suggesting that good nutrition favors the development of this pigment. The significant increases in lycopene content that accompanied nitrogen fertilization suggest a relationship between foliage growth and lycopene production. Very probably the production of lycopene in this experiment could be attributed in part, at least, to the factor of temperature. Plants with more foliage

provide more shade for developing fruits and the resulting fruit temperatures may be more nearly the optimum for lycopene production (1).

### INFLUENCE OF GAS STORAGE

The data and analysis of variance for lycopene content of the small tomato fruits ripened in air and oxygen at two different temperatures are shown in Table 2. The fruits which were stored in nitrogen at the two temperatures showed breakdown in about four days. No red or

TABLE 2

LYCOPENE CONTENT OF TOMATO FRUITS AS INFLUENCED BY STORAGE TEMPERATURE AND GAS STORAGE

| Storage Temperature | Lycopene Content* | | |
|---|---|---|---|
| | Air Storage | Oxygen Storage | Mean |
| 20° C. | 39.9 | 67.3 | 53.6 |
| 35° C. | 11.6 | 17.6 | 14.6 |
| Mean | 25.7 | 42.5 | |

* Expressed in micrograms per gram of fresh weight.

ANALYSIS OF VARIANCE

| Source of Variation | d.f. | Mean Square |
|---|---|---|
| Replications | 3 | 7.78 |
| Temperatures | 1 | 6084.00** |
| Error $a$ | 3 | 10.74 |
| Gases | 1 | 1118.90** |
| Gases × Temperatures | 1 | 453.70** |
| Error $b$ | 6 | 2.95 |

** Significant at P less than 0.01.

yellow coloration developed in these fruits, consequently, data for the nitrogen treatment has been omitted from the tables. Highly significant differences were obtained for temperatures, gases, and gases x temperatures.

Apparently the oxygen content of the atmosphere is of great importance in lycopene production. A 60 per cent oxygen level greatly increased lycopene development at both temperatures (when compared to the 20 per cent oxygen of air). An increase in the oxygen content at 35°C. resulted in increased lycopene production; however, the increase was not as great as at 20°C.

The data for xanthophyll content of the tomato fruits in the gas and temperature treatments and the analysis of variance are shown in Table 3. Significant differences were found for both. The differences

between temperatures were not as great for xanthophyll as they were for lycopene, especially for the oxygen treatment. From this observation it is not difficult to understand why tomatoes turn yellow when ripened at temperatures above 30°C., yet fail to develop much red coloring.

Apparently oxygen is a more critical factor in xanthophyll development at higher temperatures, since a greater increase was found when the oxygen content of the atmosphere was increased at 35°C. than at

TABLE 3

XANTHOPHYLL CONTENT OF TOMATO FRUITS AS INFLUENCED BY TEMPERATURE AND GAS STORAGE

| Temperature | Xanthophyll Content† | | |
| --- | --- | --- | --- |
| | Air Storage | Oxygen Storage | Mean |
| 20° C. . . . . . . . . . . . . | 3.02 | 3.57 | 3.29 |
| 35° C. . . . . . . . . . . . . | 2.39 | 3.25 | 2.82 |
| Mean. . . . . . . . . . . . . | 2.70 | 3.41 | . . . . . . . . . . . . . . . . . |

ANALYSIS OF VARIANCE

| Source of Variation | d.f. | Mean Square |
| --- | --- | --- |
| Replications. . . . . . . . . . . . . . . . . . . . . . . . . . . . | 3 | 0.2563 |
| Temperatures. . . . . . . . . . . . . . . . . . . . . . . . | 1 | 0.9025 |
| Error *a*. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | 3 | 0.0636 |
| Gases. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | 1 | 2.0022** |
| Gases × Temperatures. . . . . . . . . . . . . . . . . | 1 | 0.0931 |
| Error *b*. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . | 6 | 0.0988 |

† Expressed in micrograms per gram of fresh fruit.
** Significant at P less than 0.01.

20°C. At neither of the two temperatures did the amount of oxygen influence the development of xanthophyll to the extent that lycopene production was affected.

VARIETIES, SPECIES, AND HYBRIDS

The data on the lycopene content of tomato fruits of twelve varieties and hybrids are shown in Table 4. Rutgers was used as the standard for comparison, and the test for significance indicated that the varieties Earliana and U. S. No. 24 were significantly lower in lycopene content at the 1 per cent level, and the hybrid Rutgers x Jubilee was significantly lower at the 5 per cent level. Several other varieties and hybrids approached significance when compared to Rutgers. If Marglobe fruits had been used as the standard, many of the varieties and hybrids would have been significantly lower in lycopene content.

It is interesting to note that the wild species (*L. esculentum*), which

was included because of its apparently high pigment content, was not significantly better than Rutgers. Since Rutgers and Marglobe contained nearly the same amount of lycopene as this wild type, they should be as good sources of germ plasm for color and have the distinct advantages of greater fruit size and smoother fruit surface.

The hybrid Rutgers x Jubilee was significantly lower in lycopene content from Rutgers. According to the gene combinations (3), there should be no difference in the color of the hybrid from its red parent. However, the probable presence of a recessive tangerine gene may have caused a reduction in lycopene synthesis for the fruits of that cross.

TABLE 4

LYCOPENE CONTENT OF TOMATO FRUITS OF TWELVE
VARIETIES AND HYBRIDS

| Varieties and Hybrids | Lycopene Content* |
|---|---|
| Rutgers. . . . . . . . . . . . . . . . . . . . . . . . | 60.7 |
| Marglobe. . . . . . . . . . . . . . . . . . . . . | 63.0 |
| Pritchard. . . . . . . . . . . . . . . . . . . . . | 54.7 |
| Earliana. . . . . . . . . . . . . . . . . . . . . | 47.3 |
| Indiana-Baltimore. . . . . . . . . . . . . . . | 53.9 |
| Pan America. . . . . . . . . . . . . . . . . . . | 53.8 |
| Pritchard × Earliana. . . . . . . . . . . . . | 54.7 |
| Rutgers × Marglobe. . . . . . . . . . . . . | 56.1 |
| Rutgers × Jubilee. . . . . . . . . . . . . . . | 52.0 |
| Ind.-Baltimore × Pan America. . . . . | 53.9 |
| U.S. No. 24. . . . . . . . . . . . . . . . . . . | 48.5 |
| No. 640 (Wild L. esculentum). . . . . . | 62.1 |

* Expressed in micrograms per gram of fresh weight.
  P at less than $0.05 = 8.3$.
  P at less than $0.01 = 11.1$.

In the comparison between the Mingold and Jubilee varieties, carotene and xanthophyll values were obtained. Since the spectrophotometer was not standardized for prolycopene, this pigment was not determined. After separation of the prolycopene from carotene by chromatographic adsorption, transmission readings on a solution of this pigment were taken and are shown in Fig. 1. The transmission minimum and conversely the absorption maximum was found at 4200 A., a point considerably lower than for either carotene or lycopene.

The carotene and xanthophyll contents of fruits of the Mingold and Jubilee varieties are shown in Table 5. Significant differences (at the 1 per cent level) for both carotene and xanthophyll were found between the two varieties. This observation is interesting, since Jubilee in addition to having more of these two pigments, has a considerable quantity of the more highly colored carotenoid, prolycopene. In visual comparisons Jubilee fruit is brilliant orange and Mingold deep golden. However, the flesh colors of the two varieties show even greater differences, with Mingold having a pale yellow pulp color and Jubilee a deep orange.

E. L. DENISEN
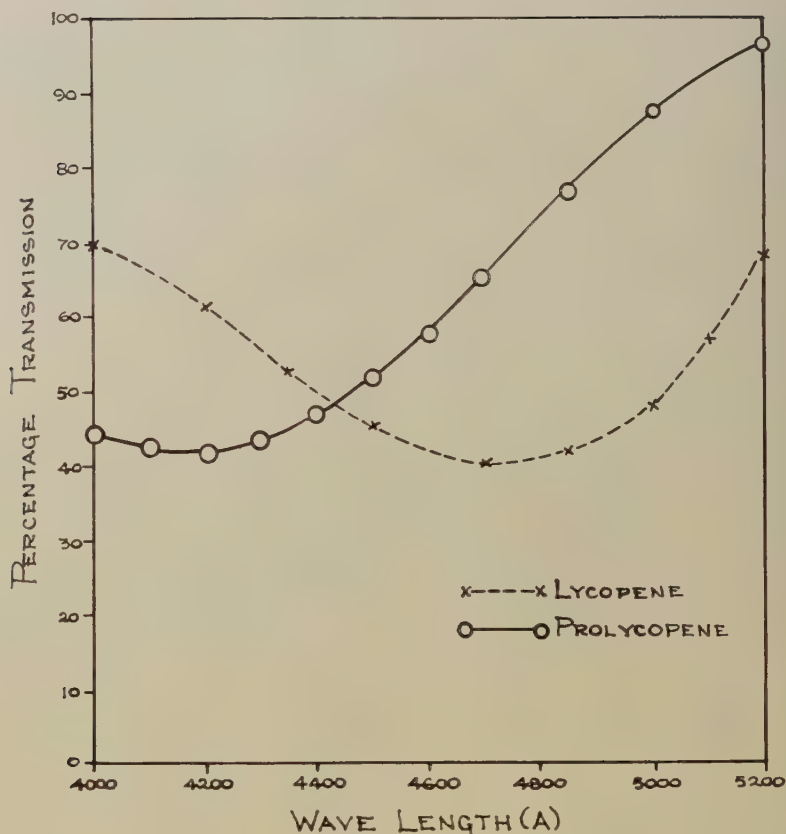


Fig. 1.—Spectral transmission curves of lycopene and prolycopene in Skelly B.

The lycopene content of the fruits of *L. esculentum, L. pimpinel-lifolium* and their interspecific cross is shown in Table 6. The small fruits of the currant tomato, *L. pimpinellifolium,* contain a very large

TABLE 5

CAROTENE AND XANTHOPHYLL CONTENT OF FRUITS OF
THE MINGOLD AND JUBILEE VARIETIES

| Varieties | Pigment Content * | |
| | Carotene | Xanthophyll |
|---|---|---|
| Mingold . . . . . . . . . . | 1.42 | 0.87 |
| Jubilee . . . . . . . . . . . | 3.98 | 1.31 |

* Expressed in micrograms per gram of fresh weight.

quantity of lycopene compared to *L. esculentum*. It is interesting to note that the interspecific cross approaches a geometric mean between its two parents with the parent of lower lycopene content exerting the greater influence.

TABLE 6

LYCOPENE CONTENT OF TOMATO FRUITS OF TWO SPECIES
AND THEIR INTERSPECIFIC CROSS

| Species or Hybrid | Lycopene Content* |
|---|---|
| *L. esculentum* (Rutgers)............ | 60.9 |
| *L. pimpinellifolium* (Acc. 160)...... | 189.2 |
| Rutgers × Acc. 160............. | 97.5 |

* Expressed in micrograms per gram of fresh weight.

## SUMMARY

1. Nitrogen fertilizer gave a significant increase in the lycopene content of tomato fruits.

2. The highest lycopene content in the plant nutrient experiment was obtained when plants were supplied with a complete fertilizer.

3. In artificially ripened fruits the most rapid development of color occurred at 20°C. in an atmosphere containing 60 per cent oxygen. Oxygen content had a greater influence than temperature in the development of xanthophyll.

4. None of twelve varieties and hybrids studied was significantly higher in lycopene content than Rutgers; however, fruits of Earliana, U. S. No. 24, and the hybrid, Rutgers x Jubilee, were significantly lower. The probable presence of a recessive tangerine gene in the Rutgers x Jubilee hybrid may have caused a reduction in lycopene synthesis for the fruits of that cross.

5. Fruits of the orange Jubilee variety were found to have a higher content of both carotene and xanthophyll than the yellow variety, Mingold. Prolycopene was identified in the Jubliee fruits by chromatographic separation and absorption spectra.

6. In a comparison of *L. esculentum* (Rutgers), *L. pimpinellifolium* (Accession 160) and their interspecific hybrid, the lycopene content of the hybrid approached a geometric mean between the two species with the tendency toward the less highly pigmented Rutgers parent.

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# EFFECT OF DIFFERENT ANTIBIOTICS ON GROWING-FATTENING SWINE[1,2]

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In 1949 Lepley (1) demonstrated that 0.25 per cent of a dried whole aureomycin mash containing 7.7 grams of aureomycin per pound increased growth rate significantly ($P = 0.01$) when added to either an all-plant basal ration or one which contained 2.5 or 5.0 per cent meat and bone scraps.

Jukes (2) *et al.* added 100 milligrams of aureomycin per pound of ration for weanling pigs. This level of aureomycin increased the average daily gain from 0.38 to 0.87 pound per day. Luecke (3) reported similar results using streptomycin.

Catron and co-workers (4), studying the relationship of nutrition to enteritis, reported significantly faster gains when either 20 milligrams of aureomycin hydrochloride or 100 milligrams of streptomycin (calculated from streptomycin sulfate) were included in an all-plant ration with or without vitamin $B_{12}$ for weanling pigs. This agrees with the work of Carpenter (5), who added 12.5 milligrams of aureomycin hydrochloride to a ration containing 10 per cent tankage and increased the average daily gains of unthrifty pigs from 0.46 to 0.88 pound per day. However, Speer *et al.* (6) failed to increase the growth rate of healthy, previously well-fed pigs under clean conditions when either 5 or 10 milligrams of aureomycin hydrochloride were included per pound of ration.

Catron *et al.* (7) fed 120 pigs different levels of aureomycin hydrochloride with and without crystalline vitamin $B_{12}$. They reported that pigs fed 5 milligrams of aureomycin hydrochloride per pound of total ration gained 0.16 pound per day faster (significant at $P = 0.01$) than

the basal pigs. The average daily gains of pigs fed 5 milligrams of aureomycin hydrochloride per pound of ration were almost as rapid as the pigs which received 40 milligrams per pound of ration. Speer *et al.* (8) reported similar results when 2 or more milligrams of procaine penicillin G were added per pound to an all-plant basal ration with crystalline vitamin $B_{12}$ added.

## EXPERIMENTAL PROCEDURE

The purpose of this experiment was to evaluate the effects of six different antibiotics when they were added individually (at 10 milligrams per pound of ration) to a good ration for weanling pigs.

Seventy weaned Duroc pigs whose average age and weight were 59.5 days and 32.7 pounds, respectively, were randomly allotted to seven different treatments by outcome groups. The outcome groups were based on litter, breeding, previous treatment, condition, sex, and weight. At the start of the experiment, all pigs were wormed with sodium fluoride and sprayed with benzene hexachloride. They were housed on concrete

TABLE 1

COMPOSITION AND CALCULATED ANALYSIS OF THE BASAL RATIONS*

|  | (Start to 75 lb.) | (75 lb. to Final Wt.) |
|---|---|---|
| Protein Level (per cent) | 18 | 15 |
| *Ingredients:* |  |  |
| Ground yellow corn | 71.0 | 78.5 |
| Solvent SBOM (blended) | 21.5 | 14.0 |
| Vitamin premix† | 2.0 | 2.0 |
| Crystalline vitamin $B_{12}$ premix‡ | 2.0 | 2.0 |
| Special steamed bonemeal | 2.2 | 2.2 |
| Iodized calcium carbonate | 0.7 | 0.7 |
| Iodized salt | 0.5 | 0.5 |
| Trace minerals§ | 0.1 | 0.1 |
| Total | 100.0 | 100.0 |
| *Calculated Analysis:* |  |  |
| Protein, per cent | 18.03 | 14.9 |
| Calcium, per cent | 0.93 | 0.91 |
| Phosphorus, per cent | 0.63 | 0.38 |
| Vitamin A, I.U. | 2075 | 2262 |
| Vitamin $D_2$, U.S.P. units | 400 | 400 |
| Riboflavin, mg. per pound | 1.70 | 1.6 |
| Niacin, mg. per pound | 18.60 | 18.5 |
| Pantothenic acid, mg. per pound | 8.00 | 7.6 |
| Choline, mg. per pound | 749 | 659 |

* Antibiotics were premixed in solvent soybean oil meal.

† Contained 250 mg. calcium pantothenate, 12.5 gm. choline chloride, 25 mg. folic acid, 500 mg. niacin, 75 mg. pyridoxine, 50 mg. riboflavin, 75 mg. thiamin, 1.5 gm. vitamin A (10,000 I.U./gm.) and 2.27 gm. vitamin $D_2$ (4 million U.S.P. units per pound) and 0.96 pound of solvent soybean oil meal per pound of premix.

‡ Contained 0.5 mg. crystalline vitamin $B_{12}$ per pound of soybean oil meal premix.

§ Added per lb. of ration (ppm): Fe-70, Cu-4.8, Co-1.6, Mn-59, Zn-4.4, and K-7.6 on $CaCo_3$ carrier.

floors and had access to outside concrete runs. The pigs were self-fed a complete ration, and water was offered ad libitum. The pigs were individually weighed, and the feed consumption by lots was recorded every two weeks. The experiment was conducted until each lot of pigs averaged 135 pounds.

The basal ration fed to Lot I consisted of ground yellow corn, blended solvent soybean oil meal, minerals (including trace minerals), vitamin A and $D_2$, and eight B-vitamin including crystalline $B_{12}$, calcium pantothenate, choline chloride, folic acid, niacin, pyridoxine, riboflavin, and thiamin (Table 1). The ration was calculated to contain 18 per cent protein until the pigs averaged 75 pounds, at which time the protein was lowered to 15 per cent by increasing the corn and decreasing the soybean oil meal.

Lots 2 through 7 received respectively 10 milligrams per pound of total ration one of the following antibiotics: aureomycin hydrochloride, streptomycin (from streptomycin sulfate), terramycin (from terramycin hydrochloride), procaine penicillin G, bacitracin, or polymixin B sulfate. As the first lot of pigs reached an average weight of 135 pounds, individual fecal samples were collected from all the pigs remaining on the experiment. The samples were quick frozen ($-23°$C.) and then shipped to the laboratory[3] for total anaerobe counts.

The anaerobe counts were made following the usual bacteriological procedure. One-half gram sample of the feces was suspended in sterile water by shaking vigorously 15 minutes. The sample was then diluted out to final concentration of one to ten thousand. One milliliter of final suspension was inoculated into 20 milliliters of agar containing 2 per cent nutrient agar and 1 per cent dextrose, run in anaerobic jars and incubated two days at $37°$C. Colonies which developed were counted visually.

### DISCUSSION

The pigs in all lots exhibited a characteristic enteritis for the first week to ten days on experiment. Thereafter the pigs receiving either aureomycin or terramycin had normal feces. All the pigs fed penicillin ceased scouring about this same time except for one pig which had diarrhea for nine weeks. The pigs receiving bacitracin scoured slightly for over two months; however, the severity of scouring here was not as great as in the pigs which received the basal ration or the basal plus either streptomycin or polymixin.

Six of the seventy starting pigs died during the course of the experiment. These pigs were diagnosed by the College Veterinary Diagnostic Laboratory; all six showed symptoms of a severe enteritis.

The survival, growth, and feed data are summarized in Table 2. Five of the outcome groups were complete at the end of the expriment; otherwise some of the pigs had died in the other five groups. Be-

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[3] These counts were made through the courtesy of Chas. Pfizer and Co., Inc., Brooklyn, New York.

cause of this, only the average daily gains from the outcome groups where all pigs survived are presented (Table 2). The average daily feed per pig and feed required per 100 pounds of gain are for all the outcome groups whether complete or not.

These data show that aureomycin and terramycin increased the gains by more than 0.20 pound over the basal ration. These differences

TABLE 2

EFFECT OF VARIOUS ANTIBIOTICS ON SURVIVAL, GROWTH AND FEED CONSUMPTION OF GROWING-FATTENING PIGS

| Antibiotic Added To Basal Ration (10 mg. per lb.) | No. Pigs* Finishing | Av.† Daily Gain | Av. Daily Feed per Pig | Feed per 100 lbs. Gain |
|---|---|---|---|---|
| None | 9 | 1.46 | 4.4 | 323 |
| Aureomycin | 10 | 1.67 | 4.8 | 298 |
| Streptomycin | 10 | 1.43 | 4.1 | 327 |
| Terramycin | 10 | 1.65 | 5.2 | 322 |
| Penicillin | 7 | 1.58 | 4.4 | 329 |
| Bacitracin | 8 | 1.46 | 4.7 | 333 |
| Polymixin | 10 | 1.40 | 4.1 | 324 |

* Ten pigs started per lot.

† Average daily gains are for the five complete outcome groups or litters; average initial weight per pig was 40 pounds and average final weight per pig 153 pounds.

only approached significance at $P = 0.05$; a difference of 0.24 pound per day would be necessary for significance at $P = 0.05$ and 0.33 pound at $P = 0.01$. This large difference required for significance is because of the small number of complete outcome groups (five) and the lack of replications. The addition of 10 milligrams per pound of ration of penicillin also increased average daily gains but not significantly under these conditions. Neither bacitracin, polymixin, nor streptomycin increased the average daily gains of the pigs on this experiment. Research workers (3,4) have demonstrated increased growth rate of pigs by the addition of streptomycin to a ration. However, the levels fed were higher than those used here.

The results of the anaerobe counts are summarized in Table 3. As some of the samples had started to thaw when received at the laboratory and anaerobe counts could not be made, all of the pigs are not represented. Aureomycin, streptomycin, terramycin, bacitracin, and polymixin significantly decreased the anaerobe count (at $P = 0.01$ for the first four and at $P = 0.05$ for the fifth). Penicillin did not influence the count. These results do not correlate with the scouring or average daily gains of pigs fed the various rations. The per cent reduction in count was compartively low; therefore, it is somewhat doubtful if the physiologic effects due to anaerobes (if any) were much influenced by the reduction.

TABLE 3

Effect of Feeding Various Antibiotics to Swine on
Anaerobe Counts of Feces

| Antibiotic Added To Basal Ration (10 mg. per lb.) | No. of Samples | Av. Anaerobe Count (per ml.) |
|---|---|---|
| None............................ | 9 | 4,333,000 |
| Aureomycin.................... | 5 | 992,000 ** |
| Streptomycin.................. | 9 | 481,000 ** |
| Terramycin.................... | 6 | 810,000 ** |
| Penicillin..................... | 7 | 3,917,000 |
| Bacitracin..................... | 6 | 810,000 ** |
| Polymixin..................... | 8 | 2,272,000 * |

\* Significant at P = 0.05.
\*\* Significant at P = 0.01.

## SUMMARY AND CONCLUSIONS

Weaned Duroc pigs in concrete drylot from weaning to approximately 135 pounds were fed either aureomycin hydrochloride, terramycin (from terramycin hydrochloride), streptomycin (from streptomycin sulfate), procaine penicillin G, bacitracin, or polymixin B sulfate at the rate of 10 milligrams per pound of total ration. Under the conditions of this experiment aureomycin, terramycin, and penicillin increased the average daily gains over the basal ration but streptomycin, bacitracin, and polymixin did not. The three antibiotics which produced the fastest gains also controlled scouring resulting from an enteritis. The control of enteritis and scouring was probably the main factor which enabled the pigs to grow faster.

Total anaerobe counts could not be correlated with scouring or rate of gain. The authors believe that the total anaerobe count is not associated with the mode of action of antibiotics.

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# SECONDARY PLANT SUCCESSION ON AN ERODED LINDLEY SOIL AS AFFECTED BY VARIATIONS IN CULTURAL TREATMENT[1]

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Plants have a modifying effect upon the soil through the addition of organic matter and the improvement of structure. They prevent erosion by protecting the surface soil, binding the soil with their roots, and maintaining an adequate infiltration capacity. These factors are important in plant development. As plant succession advances, more luxuriant and complex vegetation appears concurrently with an improvement in site quality. It is this amelioration in site conditions through the effects of vegetation that finds useful application in the rejuvenation of over-cropped and seriously eroded lands.

Ill-adapted cropping systems and overgrazing on the hilly land of southern Iowa have resulted in serious depletion of soil resources. Much of the top soil on the steeper slopes has been lost, causing a decrease in crop yields and making rotation cropping no longer feasible. It is apparent that if these conditions are to be remedied, ecologically adapted grasses and legumes should be introduced to effect successional change in the most practical and economic manner. Such plants bring about rapid and beneficial changes in the soil, while affording adequate protection against erosion. The plan in general is a modification of plant succession wherein economic cover plants are substituted for the naturally-occurring plant community, thus shortening the time when the land again can be fitted into a stable agricultural economy. The purpose of this paper is the presentation and evaluation of data obtained from experiments on cultural modifications of secondary plant succession on an eroded Lindley soil.

In a study of the stages of the succession as related to soil conditions, Campbell (9) found that the occurrence and plant composition of each stage in the succession was largely determined by the type of soil. Each stage contributed to building up humus and the water-holding capacity of the soil, thus improving the site for the following stage. Where the top soil had been removed by erosion, Sinclair and Sampson (26) observed that succession was greatly retarded if not actually prevented. They were of the opinion that under such conditions it would be erroneous to attempt establishment of a perennial grass cover without preliminary

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conditioning of the soil. Aikman (1), investigating primary plant suc-
cession in relation to erosion, determined that organic matter content
and water-holding capacity were increased with each successional ad-
vance chiefly because of increased depth of top soil.

Water content of the soil was found to be a critical factor in
vegetational change by Booth (5) in an investigation of abandoned
fields in Kansas and Oklahoma. It was observed that where contouring
and terracing were employed to trap runoff the rate of succession was
accelerated. Warner (30) obtained similar results from basin listing
on eroded southern Iowa soils. In a study of three successional weed
stages in southern Iowa, Ward (28) concluded that water-holding
capacity and available moisture of the soil were lowest in the first stage,
intermediate in the second, and highest in the third.

Clements (10) ranked improvement in structure as the most
important change in the soil during plant succession. Structure was
recognized by Russell (25) as having both direct and indirect effects
upon soil as a plant habitat; directly it affects root penetration, aeration,
and intake and conservation of water; indirectly it increases soil fertility
by influencing biological and chemical reactions. Greene and Snow (13)
observed that the physical property of the soil most directly related
to fertility was its capacity to store water for plant use without obstruct-
ing aeration. McGeorge and Breazeale (18) indicated that water was
not easily available to plants unless structure was favorable to aeration
and root penetration.

A statement concerning plant succession in relation to erosion
control and soil improvement was given by Boatman (4) in 1938. It
was noted that on most soils the natural environment had been
changed to the extent that unstable soil conditions had developed. Two
general approaches to treatment were given. One was to retire the
land and let regeneration of the site take place through a prolonged
period of natural succession. The other plan was to shorten the ecologic
succession by various cultural treatments in soil rehabilitation.

In North Carolina, Kittredge (15) observed that shortleaf pine was
benefited by a stand of sericean lespedeza which grew well under the
canopy. This plant as well as other perennial legumes was advocated
by Pieters (22) for improving soil structure and increasing fertility. It
was shown that a field of three-year sericean lespedeza, when plowed
under and planted to corn, produced 60.5 bushels per acre, whereas
on similar untreated land the yield was 25 bushels. In erosion control
this legume was extremely beneficial. Eroded fields with severe gullying
were seeded, and in the course of four years erosion was no longer a
problem. In testing twenty legumes for site adaptation on eroded
southern Iowa soils, Brewer (7) found that birdsfoot trefoil, crown
vetch, and sericean lespedeza showed the best response among the
perennials.

Culbertson (11) found birdsfoot trefoil to be a promising legume
in rebuilding poor soils. In eastern New York it was found growing

luxuriantly on soils deficient in lime, in association with goldenrod, devil's paintbrush, and poverty grass. It was described by McKee and Schoth (20) as a deep-rooted perennial adapted to poor soils. European writers indicated its drouth resistance and adaptability to conditions under which clover fails. Based on experiments at Rothamstead, Robinson (24) reported the efficacy of planting birdsfoot trefoil on soils low in organic matter. Its beneficial effects in rebuilding structure were proved by Ward (29) in eroded southern Iowa soils.

Russell (25), reviewing Russian work, reported that two to three years of grass growth were necessary before structural improvement became noticeable. McHenry and Newell (19) showed the influence of various grasses on the organic matter content and structure of a fine textured soil. They found a close relationship between soil aggregation and total nitrogen and organic matter. Native grasses were effective in crumb structure formation according to Martin (17). These results were not observed in soils low in colloids, indicating the basic dependence of crumb formation on the clay content of the soil (17, 29).

A number of methods have been devised to evaluate structure through the measurement of its characteristic features, including the size distribution and amount of the soil aggregates. The percentage and size of aggregates largely determine the extent of the large pores, which Schumacher called noncapillary pores. Nelson and Baver (21) observed a better correlation between pores drained at 40 centimeters of tension and percolation than at any other tension. This tension was used by Wilson and co-workers (32) in calculating noncapillary porosity of several Iowa soils. Leamer and Shaw (16) described a simple apparatus for measuring noncapillary porosity on an extensive scale. It was found by Bradfield and Jamison (6) that the noncapillary pores were responsible for drainage, percolation, and aeration of soils.

Tiulin (27) used a graded nest of screens in determining aggregate sizes and suggested that soil particles greater than 0.25 millimeter were responsible for favorable structure. Yoder (33) pointed out the limitations of the elutriator as imposed by Stokes' law, and proposed a direct method of determining water stable aggregates by wet sieving. Browning and co-workers (8) used the "coefficient of aggregation" in expressing soil aggregation.

## DESCRIPTION OF FIELD SITES AND EXPERIMENTAL METHODS

A representative area of Lindley silt loam at the Experiment Station near Floris, in Davis County, Iowa, was selected for study of the effects of successive vegetative covers upon soil improvement. The Lindley soil, derived from Kansas till, is generally described as light colored, medium textured, acid, and slowly permeable. Erosion is severe and the remaining top soil varies from 0 to 8 inches in depth. The subsoil is a gravelly, silt loam between depths of 8 and 30 inches. Below 30 inches the till is strongly mottled.

Previous research (7) had indicated various physical and chemical

differences within the area, on which basis it was divided into several sampling sites. The present investigation was concerned with two of these sites designated as L-1 and L-3. These symbols will serve to designate the two sites through the remainder of this paper. A summary of soil characteristics determined in 1939 is given in Table 1. The approximate location of the two experimental sites is presented in Fig. 1.

The field under study was abandoned in 1933. By 1936 the vegetation had advanced to the ragweed stage in which *Ambrosia elatior* was

TABLE 1

Summary of Some Chemical and Physical Characteristics of the Two Experimental Sites at Two Depths*

| Characteristic | Site L-1 | | Site L-3 | |
|---|---|---|---|---|
| | 0–6 Inches | 6–18 Inches | 0–6 Inches | 6–18 Inches |
| Per cent C†.............. | 1.00 | 0.68 | 0.89 | 0.54 |
| Per cent N†.............. | 0.069 | 0.05 | 0.07 | 0.04 |
| Total base exchange capacity‡............... | 10.62 | 17.69 | 6.96 | 9.11 |
| Percentage base saturation... | 89.10 | 60.00 | 92.0 | 59.40 |
| Exchangeable Ca‡......... | 7.93 | 6.58 | 5.65 | 3.78 |
| Average depth of top soil.... | 4–6 inches | | 2–4 inches | |
| Per cent slope............ | 4–7 | | 6–8 | |

* Data taken from Brewer (7).
† Percentages expressed on an oven-dry basis.
‡ Milli-equivalents per 100 grams of oven-dry soil.

dominant. A year later 3 tons of lime per acre was applied and a seedbed prepared for sweetclover. In the intervening years to April 1943, various grass and legume plots were laid out on the two sampling sites (Figs. 2, 3, 4, 5, and 6). Bare plots, maintained as checks, were included in the plot design. Unplowed strips between the plots were concurrently allowed to develop under natural succession during the ten-year period ending in the fall of 1947, at which time soil samples were taken from the plots. A diagram of the natural conditions and cultural modifications by years is shown in Fig. 7. The two legume and two grass species selected for study, and the dominant native species appearing in the unplowed strips are listed in Table 2.

The vegetative stages in order of occurrence on the eroded sites had previously been studied (28, 30). The nature and sequence of these plant communities are shown in Figs. 8, 9, and 10. *Plantago aristata* was an early invader of the eroded site. The subsequent invasion of *Ambrosia elatior* indicated the successional trend, to be followed by the establishment of the goldenrod-aster stage in which *Solidago nemoralis* and *Aster ericoides* were principal dominants. Weedy communities of *Erig-*

**SITE 7**
**DAVIS COUNTY**
SECS. 15,16,21, T. 70N, R13W of 5½RM
187 ACRES

SCALE 1"=400'

N

FIG. 1.—Location of experimental sites on the Hillculture Farm near Floris, Iowa, with respect to contour pattern.

eron strigosus and *Lactuca canadensis* were interspersed in the golden-rod stage. *Poa pratensis* came into the succession following these peren-nial communities, and at the time of the study represented the highest stage.

A description of the design of the experiment initiated in 1939 on site L-3 and the details of plot establishment were given by Brewer (7). The milacre plots were laid out in April, 1939, among five contour furrows. Soil preparation included shallow plowing and harrowing, after which the plots were raked by hand to provide a good seedbed. Of the legum-
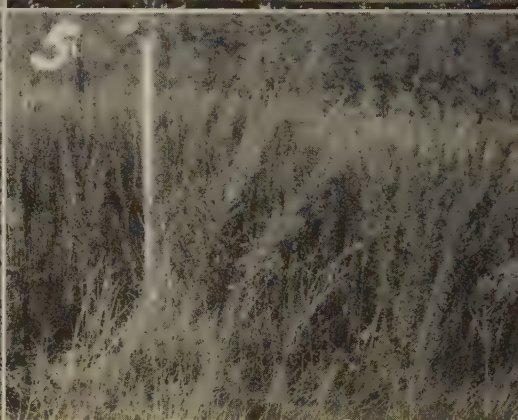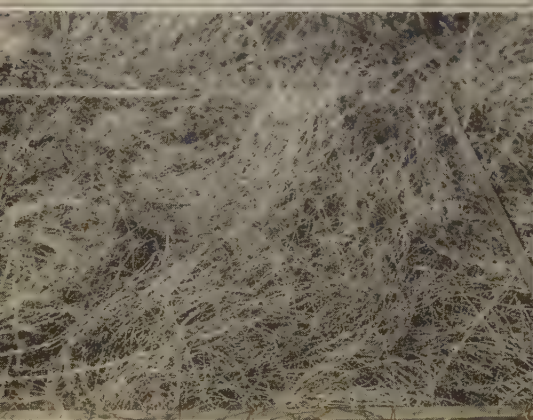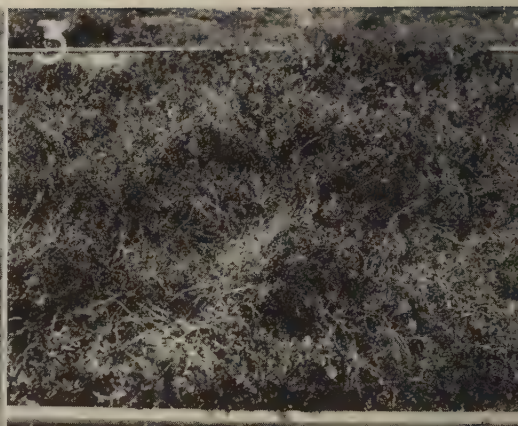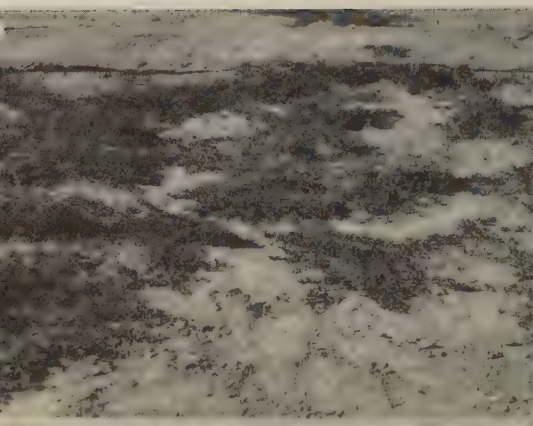
Fig. 2.—A *Lotus corniculatus* plot on site L-3 during first year.
Fig. 3.—*Lotus corniculatus* plot on site L-3 during the second year, showing increased cover.
Fig. 4.—Matted condition of *Lotus corniculatus* on site L-3 in October of eighth year of growth.
Fig. 5.—Fourth year plot of *Lespedeza sericea* on site L-1.
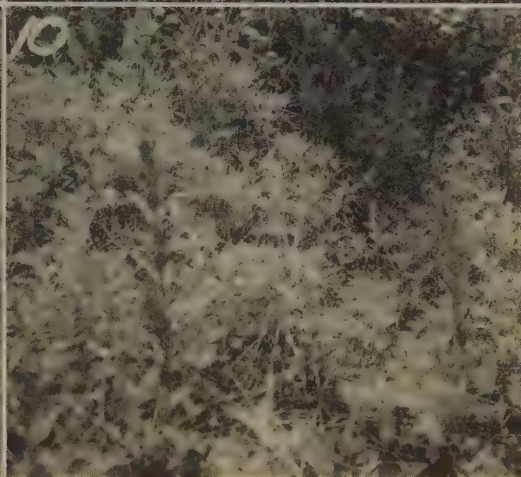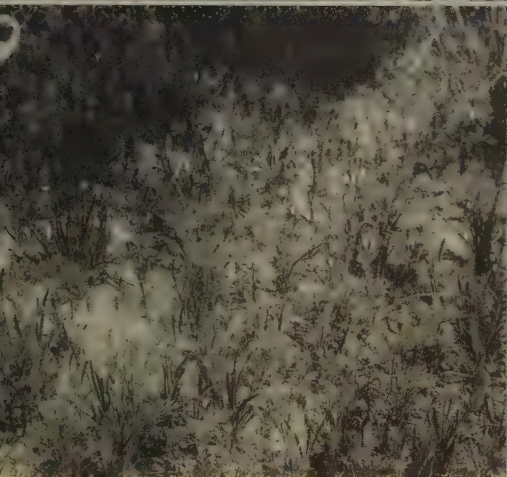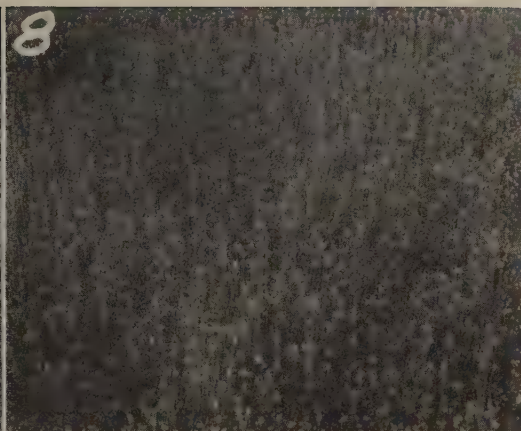
FIG. 6.—Fourth year stand of *Andropogon gerardi* on site L-1.
FIG. 8.—*Plantago aristata* community on eroded site after aggregation.
FIG. 9.—Invading *Ambrosia elatior* in a *Plantago aristata* community.
FIG. 10.—Invading *Solidago nemoralis* in an *Ambrosia elatior* community.

inous species planted in 1939, only *Lotus corniculatus* was used in a soil comparison study with the goldenrod-aster stage which occurred in the unplowed strip. The dominant species of this stage were *Solidago nemoralis* and *Aster ericoides.*

On site L-1, various grasses and legumes were sown in April, 1943, following four till crops. The milacre plots occupied three contour benches, as it was believed less soil variation existed at the same level

TABLE 2

SPECIES OF PLANTS OF THE STAGES OF MODIFIED AND UNMODIFIED PLANT SUCCESSION

| Scientific Name* | Common Name |
|---|---|
| *Andropogon gerardi* Vitman | Big bluestem |
| *Dactylis glomerata* L. | Orchard grass |
| *Lespedeza sericea* (Thunb.) Benth.† | Sericea lespedeza |
| *Lotus corniculatus* L. | Birdsfoot trefoil |
| *Poa pratensis* L. | Kentucky bluegrass |
| *Solidago nemoralis* Ait. | Field goldenrod |
| *Aster ericoides* L.‡ | Heath aster |
| *Erigeron strigosus* Muhl.§ | Daisy fleabane |
| *Lactuca canadensis* L. | Wild lettuce |
| *Ambrosia elatior* L.‖ | Small ragweed |
| *Plantago aristata* Michx. | Bracted plantain |

* Scientific names of plants as used in this thesis are in accord with those in recent manuals by Deam (12) and Jones (14).

† *Lespedeza cuneata* Dum. de Cours according to strict taxonomy, but *L. sericea* used here because of universal use in this country.

‡ *A. multiflorus* Ait. of Gray's New Manual of Botany. 7th edition (23).

§ *E. ramosus* (Walt.) B. S. P.; not *E. ramosus* Raf.

‖ Including *A. artemisiifolia* L.

around the slope. Among the cover plants seeded in 1943, *Lespedeza seciera, Andropogon gerardi,* and *Dactylis glomerata* were tested for their effects upon soil structure. Bare plots kept free of invading vegetation were used as checks in both sampling areas.

Representative soil samples were obtained at random from the treatment plots on L-1 and L-3 for the evaluation of soil structure. Four replicates were sampled for aggregate analysis and productivity tests at the 0- to 6-inch and 6- to 12-inch depths. These samples were collected in paper bags and later spread on a flat surface to dry. Large clods were crumbled with the fingers before drying left them excessively hard and unusable. Six undisturbed samples were collected from each of two plots at the 0- to 3-inch depth for permeability and noncapillary porosity determinations. A Coile sampler was used to obtain the undisturbed core samples, which were collected in metal soil boxes and later allowed to air dry before using.

Several methods for measuring physical properties as indices of soil structural conditions under the various treatments seemed desirable. Any one approach alone might not provide conclusive results, whereas several, showing consistent findings, would provide more reliable data.
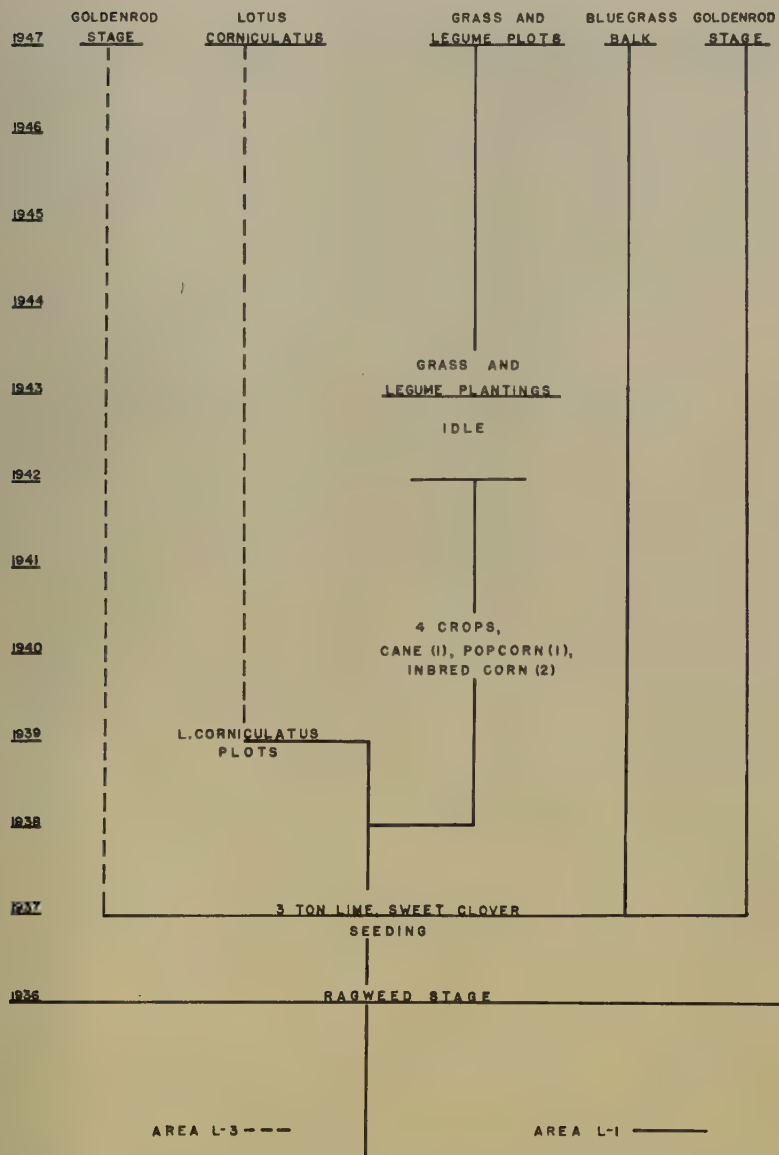
FIG. 7.—Comparison of natural and modified secondary plant succession on eroded Lindley silt loam.

## AGGREGATE ANALYSIS

The percentage of water stable aggregates was determined by the wet sieve method described by Yoder (33). Duplicate 50-gram samples of air dried soil were randomly drawn from a quartered section of the plot sample. The percentages of water stable aggregates greater than 0.25 millimeter and 2.00 millimeters were measured. Various workers have used values based on percentages of aggregates greater than 0.25 millimeter, although recently it has been reported that size aggregates larger than 2.00 millimeters are most closely related to structural stability (31). The data presented herein are expressed on the basis of aggregates 2.00 millimeters or larger.

### PERMEABILITY AND NONCAPILLARY POROSITY DETERMINATIONS

The undisturbed samples obtained with the Coile sampler were used in determining relative degree of permeability and percentage of noncapillary pores. Six samples were measured simultaneously by first saturating for 24 hours, after which the samples were placed on a plate apparatus described by Leamer and Shaw (16). A piece of cheesecloth was attached to the bottom of each sample by means of a rubber band to prevent any soil loss due to slaking. A leveling bottle connected to the plate by rubber tubing was placed 40 centimeters below the plane of the soil samples. An open-top bell jar was placed over the samples to reduce evaporation. After 24 hours the samples were removed and weighed.

Before oven-drying to determine the noncapillary porosity, the samples were placed in a permeameter to measure permeability. A constant head of 1 centimeter was maintained and readings were taken for 5-minute periods. A mean value was obtained from a series of readings for each sample expressed as centimeters per minute per centimeter head. The samples were then oven-dried and weighed. Total volumes of soil and air were calculated using 2.65 as the specific gravity of the dried soil. The difference in volume as measured by the displaced capillary water upon drying and the total pore space indicates noncapillary porosity. The tension of 40 centimeters of water for determining the noncapillary pore space of soils has been used by several workers (3, 27, 32).

## EXPERIMENTAL RESULTS

### AGGREGATE ANALYSIS

The mean values of the percentage of water stable aggregates greater than 2.00 millimeters of soil samples from site L-1 are presented in Table 3. Statistical analysis of the data indicated highly significant differences among treatments for the 0- to 6-inch depth, and significant differences at the 5 per cent level for the lower depth. The highest percentages for both depths were indicated in soil samples from plots of *Lespedeza sericea*. A sharp decrease in percentages for all treatments with the exception of the bare plot is apparent for the 6- to 12-inch

depth, as well as a narrower range of differences among treatments compared to the 0- to 6-inch depth. Depths and the depth x treatment interaction also were significantly different.

The results of aggregate analysis of soil samples from plots of site L-3 are shown in Table 4. Differences in plot treatments at the 0- to 6-inch depth were highly significant, but those at the lower depth were

TABLE 3

THE PERCENTAGE OF WATER-STABLE AGGREGATES GREATER THAN 2.00 MILLIMETERS AT TWO SOIL DEPTHS FROM PLOTS ON SITE L-1 AND RESULTS OF ANALYZED DATA

| Kind of Cover | Percentage Stable Aggregates Greater Than 2.00 Mm. at | |
|---|---|---|
| | 0–6 Inches | 6–12 Inches |
| Lespedeza sericea* | 18.4 | 8.7 |
| Andropogon gerardi† | 11.5 | 4.6 |
| Dactylis glomerata* | 7.3 | 4.3 |
| Bluegrass balk* | 15.6 | 5.3 |
| Goldenrod site* | 12.2 | 8.6 |
| Check plot (bare) † | 5.3 | 6.8 |

* Mean values of samples from milacre plots replicated four times.
† Treatment and depth differences significant at the 1 per cent level; treatment depth interaction significant at the 5 per cent level.

not significant. *Lotus corniculatus* was highly effective in increasing the percentage of aggregates at the 0- to 6-inch depth compared to the goldenrod cover. Depths and the depth x treatment interaction were also significantly different.

DEGREE OF PERMEABILITY

Permeability constants for the 0- to 3-inch depth for treatment plots of site L-1 are presented in Table 5. The ten-year balks of *Poa pratensis* and goldenrod were superior to the four-year plant treatments, *Lespedeza sericea, Andropogon gerardi,* and *Dactylis glomerata,* although

TABLE 4

THE PERCENTAGE OF WATER-STABLE AGGREGATES GREATER THAN 2.00 MILLIMETERS AT TWO SOIL DEPTHS FROM PLOTS ON SITE L-3 AND RESULTS OF ANALYZED DATA

| Kind of Cover | Percentage Stable Aggregates Greater Than 2.00 Mm. at | |
|---|---|---|
| | 0–6 Inches | 6–12 Inches |
| Lotus corniculatus* | 18.0 | 7.2 |
| Goldenrod site* | 11.1 | 5.0 |
| Check plot (bare) † | 4.5 | 7.2 |

* Mean values of samples from milacre plots replicated four times.
† Treatment differences significant at the 5 per cent level; depth differences and depth treatment interaction significant at the 1 per cent level.

$t$ values were not significant in comparing *L. sericea* and *A. gerardi* with *P. pratensis*. A treatment comparison of *P. pratensis* and *D. glomerata* showed significance at the 5 per cent level. All vegetative covers significantly increased the permeability rate compared to the bare plot. An analysis of group data between two treatments was used in these tests.

TABLE 5

Permeability Constants and Noncapillary Porosity for the 0–3 Inch Depth of Soil From Plots on L-1 and Analysis of Group Data

| Kind of Cover | Permeability Constant in Mls–Min–Cm Head | Noncapillary Porosity by Volume (40 Cm. Tension) |
|---|---|---|
| *Lespedeza sericea* † . . . . . . . . . . . . . . . | 0.90 | 8.6 |
| *Andropogon gerardi* † . . . . . . . . . . . . . | 0.96 | 8.4 |
| *Dactylis glomerata* † . . . . . . . . . . . . . | 0.81 | 6.1 |
| *Poa pratensis* † . . . . . . . . . . . . . . . . . | 1.23 | 11.9 |
| Goldenrod site † . . . . . . . . . . . . . . . | 1.68 | 7.8 |
| Check plot (bare) ‡ . . . . . . . . . . . . . | 0.24 | 4.8 |

† Mean values of triplicate samples from each of two plots.
‡ For comparison only.

| Treatment Comparison | $t$ = values with 22 degrees of freedom | |
|---|---|---|
| | Permeability Constant | Noncapillary Porosity |
| *Poa pratensis* . . . . . . . . . . . . . . . . . . . *Lespedeza sericea* . . . . . . . . . . . . . . . . | 1.987 | 2.006 |
| *Poa pratensis* . . . . . . . . . . . . . . . . . . . *Andropogon gerardi* . . . . . . . . . . . . . . . | 1.712 | 1.912 |
| *Poa pratensis* . . . . . . . . . . . . . . . . . . . Check plot . . . . . . . . . . . . . . . . . . . . | 7.071 ** | 3.290 ** |
| *Poa pratensis* . . . . . . . . . . . . . . . . . . . *Dactylis glomerata* . . . . . . . . . . . . . . . | 2.320 * | 3.290 ** |
| *Dactylis glomerata* . . . . . . . . . . . . . . . Check plot . . . . . . . . . . . . . . . . . . | 9.047 ** | 1.171 |
| Goldenrod site . . . . . . . . . . . . . . . . heck plot . . . . . . . . . . . . . . . . . . . . C | 7.119 ** | 1.935 |

* 5 per cent level of significance.
** 1 per cent level of significance.

The results of permeability determinations in the 0- to 3-inch depth for treatment plots on L-3 are presented in Table 6. Both the naturally-occurring goldenrod community and the *Lotus corniculatus* significantly increased the permeability rates in comparison with the bare plots, although no significance was found between them.

## NONCAPILLARY POROSITY

Results of noncapillary porosity determinations for the L-1 treatments are shown in Table 5. *Poa pratensis* was superior to the other treatments. Porosity values for *Andropogon gerardi* and *Lespedeza sericea,* however, were not significantly different when compared to *P. pratensis*, despite the six-year difference in occupancy. *Dactylis glomerata* had not significantly increased noncapillary porosity over the goldenrod site or the bare plot. On site L-3, *Lotus corniculatus* was superior to the goldenrod community in improving noncapillary porosity. The native community did not significantly alter porosity in comparison with the bare plot (Table 6).

TABLE 6

PERMEABILITY CONSTANTS AND NONCAPILLARY POROSITY FOR THE 0–3 INCH DEPTH OF SOIL FROM PLOTS ON L-3 AND ANALYSIS OF GROUP DATA

| Kind of Cover | Permeability Constant in Mls–Min–Cm Head | Noncapillary Porosity by Volume (40 Cm. Tension) |
|---|---|---|
| *Lotus corniculatus* †. . . . . . . . . . . . . . | 0.93 | 9.4 |
| Goldenrod site †. . . . . . . . . . . . . . | 0.81 | 5.8 |
| Check plot (bare) ‡. . . . . . . . . . . . | 0.26 | 4.8 |

† Mean values of triplicate samples from each of two plots.
‡ For comparison only.

| Treatment Comparison | $t$ = values with 22 degrees of freedom | |
|---|---|---|
| | Permeability Constant | Noncapillary Porosity |
| *Lotus corniculatus*. . . . . . . . . . . . . . . . Goldenrod site. . . . . . . . . . . . . . . | 0.706 | 3.000* |
| *Lotus corniculatus*. . . . . . . . . . . . . . . . Check plot. . . . . . . . . . . . . . . . . . | 5.448** | 3.833** |
| Goldenrod site. . . . . . . . . . . . . . . Check plot. . . . . . . . . . . . . . .   . . . . | 4.552** | 0.714 |

* 5 per cent level of significance.
** 1 per cent level of significance.

## DISCUSSION

Vegetative change and rate of soil improvement in secondary plant succession on abandoned lands are at best slow, and require many years before fertility and physical conditions of the soil are restored to their former degree. The agriculturist is interested in reclaiming waste land as rapidly and economically as possible. In hilly, poorly adapted cropping areas as in southern Iowa, the establishment of vegetative covers has been used in the practical restoration of eroded soils.

In the present study, two grasses and two legumes were compared with dominant plants in the secondary plant succession. The purpose was to determine the effects of modifying the secondary plant succession by the substitution of selected plants for those naturally occurring in the stages of the succession. Physical improvement of the soil through the evaluation of structural conditions was used as a criterion in measuring the residual effects of modification.

Following ten years of development after the ragweed stage, successional endpoints on site L-1 were the bluegrass and the slightly less well-developed goldenrod-aster stages. Compared with these for structural effects upon the soil were the four-year plant covers of *Lespedeza sericea, Andropogon gerardi,* and *Dactylis glomerata.* Attention is called to Fig. 7 which shows the successional history of the site and the disposition of the experimental area prior to the establishment of these three plant covers. It is noted that, during the period from 1938 to 1942, four cultivated crops were taken from the area on which the three plant covers were later established. Although data of physical conditions as might be found between the undisturbed and cultivated sites as of the close of the 1942 season, the assumption is made that structural conditions of the cultivated soil were inferior to those of the balks of goldenrod-aster and bluegrass. Such differences in structural conditions as might be found between the undisturbed and cultivated areas should be interpreted not only in the light of this assumption, but also in view of the fact that four years of plant reaction on the treatment plots is being compared with ten years of undisturbed plant development.

The effects of plant covers upon percentage of aggregates greater than 2.00 millimeters were significantly different. Of the four-year plant cover treatments, *Lespedeza sericea* made the most favorable structural improvement in the 0- to 6-inch depth which also exceeded that of the undisturbed plant covers (Table 3). *Dactylis glomerata* was shown to be the lowest of any plant cover in its effects upon aggregation. In the 6- to 12-inch soil level, effects of all plants were sharply lower, *Lespedeza sericea* and the goldenrod-aster stage being only slightly better than the bare plot in increasing aggregation. Contrary to this decrease in aggregation at the lower depth for the plant covers, a slight increase was found for the bare plot. A possible explanation for this increase in the lower level is the deterioration of surface structure due to compaction of rain, and the leaching of colloidal material into the lower depth. Baver (2) has pointed out the importance of clay present in the soil as a factor in the formation of aggregates.

The depth x treatment interaction was significant in affecting aggregation differences, being largely due to the decrease in size aggregates for *Andropogon gerardi* and *Poa pratensis* (bluegrass balk) in the 6- to 12-inch soil level. The reason for these values could not be explained; however, the relatively short tenure of occupancy may be the reason. As stated by Martin (17) several years are necessary before effects of grasses upon aggregation can be measured.

Degree of permeability and noncapillary porosity of samples in the 0- to 3-inch depth were highest under the bluegrass balk, although these values were not significantly greater than the values for either *Lespedeza sericea* or *Andropogon gerardi*. This could indicate that *Lespedeza sericea* and *Andropogon gerardi*, particularly the former, had a favorable influence upon structure to a degree, which in four years was slightly less than that of *Poa pratensis*, the latter developed through secondary succession on soil undisturbed for a ten-year period.

Attention is called to Fig. 7 which shows the successional history of site L-3. *Lotus corniculatus* in eight years of occupancy made the better improvement in physical condition of the 0- to 6-inch soil level in comparison to the ten-year community of goldenrod-aster, in which *Solidago nemoralis* and *Aster strigosus* were principally dominant. This superiority is shown in Table 4 in which percentage of aggregates greater than 2.00 millimeters of soil sampled from plots of *Lotus corniculatus* was significantly greater than for goldenrod-aster and bare plot. The superior effects of *Lotus corniculatus* did not extend to the 6- to 12-inch level, however, where improvement in structure was no greater than in the bare plot. Using 3-inch intervals and aggregates greater than 0.25 millimeter as a basis of measurement, Ward (29) found that the effects of *Lotus corniculatus* upon structure did not extend beyond the top 9 inches of soil.

Differences in relative permeability between the *Lotus corniculatus* and goldenrod-aster soil samples on site L-3 were not significant. Noncapillary porosity values, however, were found to be significantly different. The reason for significant difference between the two treatments in one index and not in the other is not apparent since these two indices have been found to be correlative (2). Inadequate sampling for work of this type might be the explanation for discrepancy, as well as possible slaking of the soil samples which would destroy the validity of the permeability tests.

### SUMMARY

Two selected species of grasses, *Andropogon gerardi* and *Dactylis glomerata*, and two of legumes, *Lespedeza sericea* and *Lotus corniculatus*, were compared with the plants in current undisturbed stages of secondary plant succession for their effects on the rate of successional change and site improvement on eroded Lindley silt loam in southeastern Iowa.

Residual effects of the plants on the soil, measured to determine degree of successional change and site improvement, were percentage of water-stable aggregates, degree of permeability, and noncapillary porosity.

Of the four introduced plants, *Lespedeza sericea* increased soil aggregates in the top 6 inches of soil to the greatest degree and *Dactylis glomerata* had the least effect.

*Lotus corniculatus* was found to be more favorable in soil improve-

ment effects in eight years than was the naturally developing golden-rod-aster stage on the same soil in ten years.

It would seem that *Lotus corniculatus* and *Lespedeza sericea* are superior to the plants in the natural secondary plant succession in restoring the structure of eroded Lindley silt loam.

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# A DUAL PURPOSE TITRIMETER AND DISPENSER[1]

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In the course of an investigation on the synthesis of amino acids by bacteria it became apparent the large number of assay tubes required could not be prepared and titrated by conventional methods. Manual titrations led to inconclusive results and were time consuming. Automatic pipetting machines were available; however, these solved only the easier of the two problems. The apparatus described was constructed with a view to serving as a titrimeter and dispenser.[2]

## CONSTRUCTION

The titrimeter comprises three separate units: (1) a device for measuring time, (2) a pressure regulator to maintain a constant flow of acid or base, and (3) a galvanometer connected to two half-cells to indicate the end point of titration.

### TIMER

A 10-inch aluminum disk was mounted on the turntable of an electric record player $(A)$[3] with brass escutcheon pins as rivets. A ¼-inch diameter silver button $(B)$ was soft soldered to the end of a ¼ inch by 2½ inch phosphor-bronze strip. The silver acted as the contact point to the disk through a perforated mask of stencil paper which had twelve equally spaced perforations ¾ inch in diameter. The mask was secured by a lucite disk 3/16 inch thick and 6½ inches in diameter held by a brass knob, which was pressed on to the center post of the turntable. The twelve contacts per revolution of the disk were recorded on a Veeder Root magnetic counter $(C)$. Thus, the end point of any titration was indicated by the total number of counts.

A single pole single throw 10,000 ohm, 11 milliampere, General Electric plate relay $(D)$ was placed in the 110 volt circuit to activate the counter. The relay operated from a 45 volt B supply $(G)$ and was activated by the contact point on the disk. The low amperage drain of the relay reduced arcing on the disk to a minimum.

[2] A titrimeter has been constructed by M. D. Cannon and is referred to as "In press" by Henderson and Snell (2).

[3] Letters $(A)$, $(B,)$ $(a)$, $(b)$ etc. refer to schematic wiring diagram, Fig. 1.

Fig. 1.—Schematic wiring diagram of titrimeter-dispenser. Refer to text for description.

PRESSURE REGULATOR

Air line pressure was used to maintain a flow of base into the assay tubes. Pressure was roughly controlled by a Meco dual gauge gas reducing valve and pressure regulator. The air was passed through sulfuric acid and 40 per cent sodium hydroxide to remove water and $CO_2$. It was then passed through a column of Ascarite and a cotton plug filter before entering the reservoir containing base. A micro regulator was placed between the filter and the reservoir. This was constructed of glass in the form of a U tube; the inside diameter of the one arm was three times that of the other. The tube was half filled with mercury; a contact wire was placed in the smaller arm which was connected to a brass T by means of a copper tube and Tygon tubing. The free end of the contact wire was passed along the bore of the Tygon tubing and soldered to the copper tube thus completing a circuit from the copper tube to the mercury in the larger arm. The vertical arm of the brass T was fitted with a carburetor check valve in such a way that the needle valve could drop; the needle was held in place by an arm and spring. The arm was pulled downward by a solenoid when the rising mercury column made contact with a wire held at the desired level in the larger arm. The solenoid was operated by a 3-volt battery. The orifice of the check valve was filled with soft solder through which a No. 60 hole was drilled to bleed off the air more slowly and prevent rapid and large changes in pressure in the system.

The remaining horizontal opening of the T was fitted with a second T. One opening received the treated air, and the other opening supplied the reservoir. The micro regulator permitted a pressure of $\pm$ 1 millimeter of mercury to be maintained. The pressure was easily adjusted to the needs of the experimenter by changing the position of the contact wire in the open arm of the manometer.

The base used for titration was approximately 0.02 $N$ NaOH and was kept in a 20-liter pyrex bottle. The stopper was wired in place. Base was fed under pressure from the bottle through an all glass system to a solenoid valve ($E$), Guardian Electric No. 14 110 volt $AC$. This was rebuilt in such a way that the plunger drew down a brass bar ($a$) against spring compression when the coil was activated. When not activated, the brass bar ($a$) pressed against a second bar ($b$) so that a gum rubber tube passing between the two was pinched off. In this way, the flow of base could be regulated. A foot switch ($H$) was used to operate the solenoid valve and the counter; both were activated simultaneously. Thus the time of flow was recorded by the counter. The base fed through a glass capillary with a side orifice at about 45° to the horizontal axis of the capillary; the base as it entered aided in stirring the contents of the assay tubes. The capillary formed the center of the quinhydrone electrode.

### HALF-CELLS

The half-cells were of the calomel and quinhydrone type. The former was constructed as outlined by Mack and France (1934). The latter was made by wrapping a No. 26 $B$ and $S$ gauge platinum wire around the flared end of a ⅜-inch (outside diameter) glass T tube which also served as the entrance for $CO_2$-free air. Bubbles of $CO_2$-free air under pressure stirred the contents of the tubes. This arm was 9½ inches long and reached to the bottom of the assay tubes.

The electrodes were connected by a bridge of saturated KCl which fed dropwise slowly into the assay tube to maintain a clean surface at the open end of the bridge. It was found convenient to feed saturated KCl solution from a dropping funnel into the calomel half cell to replace the solution passing out through the bridge. The cells connected to a Leeds and Northrup portable pointer type galvanometer, 1 μ ampere per scale division. The galvanometer was adjusted to read zero at a $p$H of 7.1. Assay tubes were titrated to this endpoint. [Cf. Henderson and Snell (2).]

To titrate an assay tube, four drops of a saturated solution of quinhydrone were placed in the tube. This was then placed in such a way that the platinum electrode, stirring and base feed tube and salt bridge were immersed in the contents. The motor was started and the foot switch engaged. Base was fed into the assay tube until the galvanometer read zero. Passage through the zero point was rather rapid, and care had to be exercised not to overrun the endpoint.

### DISPENSER

The half-cells and counter are not required in the dispensing mechanism, otherwise the unit is the same as described above. Two changes are essential, i.e. the mask for the disk must be changed to one having a sector removed so that contact is maintained through about one-half

of the revolution of the turntable, and the counter must be removed from its circuit and the magnetic valve placed in by means of a double pole double throw switch $(F)$.

The medium to be dispensed was placed in a suitable glass bottle. Pressure was fed to the bottle from the line and the outlet for medium was connected to a gum rubber tube $(I)$ at the magnetic valve $(E)$. Three-way glass stopcocks were used to shift pressure from one bottle to another or to change the liquid fed to the magnetic valve. A length of pressure tubing fitted with a glass tip served to dispense the medium into the assay tubes. The unit amount dispensed could be varied by changing the mask, the speed of the turntable or the orifice of the dispensing tip.

## RESULTS

Standard curves for aspartic and glutamic acids are set forth in Fig. 2. The activity of the assay organism and variation among batches of media will play a part in determining the shape of the curve. Data relating to the titration of 200 assay tubes are shown in Table 1. The rapidity of the titrations and narrow range of $p$H at the end point are of interest.

TABLE 1

RATE OF TITRATION OF ASSAY TUBES WITH TITRIMETER-DISPENSER

| No. of Tubes | Average Count per Tube | Total Time (minutes*) | Time (seconds) per tube | Final $p$H (Av. of 20 tubes) | Final Range ($p$H) |
|---|---|---|---|---|---|
| 200 | 104.5 | 120 | 36 | 7.09 | 7.04–7.12 |

* Time included that for removal of tube caps and addition of quinhydrone.

The weight of base delivered as plotted against number of counts is shown in Fig. 3. No attempt was made to calibrate the orifice to deliver a unit weight per count since such a ratio depends on the speed of the disk and the pressure of the system. Speed and pressure may be adjusted as long as they remain constant for any series, since each series will be interpreted from its own standard curve.

The weight of assay medium dispensed for 10 random samples is set forth in Table 2. The narrow range of weight, 0.06 grams, and the rate, 10 tubes per minute, are important. The rate was 6.4 tubes per minute for a similar series dispensed manually from a 10 milliliter pipette at a volume of 3 milliliters per tube. The weight ranged from 2.89 to 3.09 grams.

## SUMMARY

A dual purpose titrimeter-dispenser has been described. Speed and accuracy of titrations and filling of assay tubes have been greatly increased by its use.
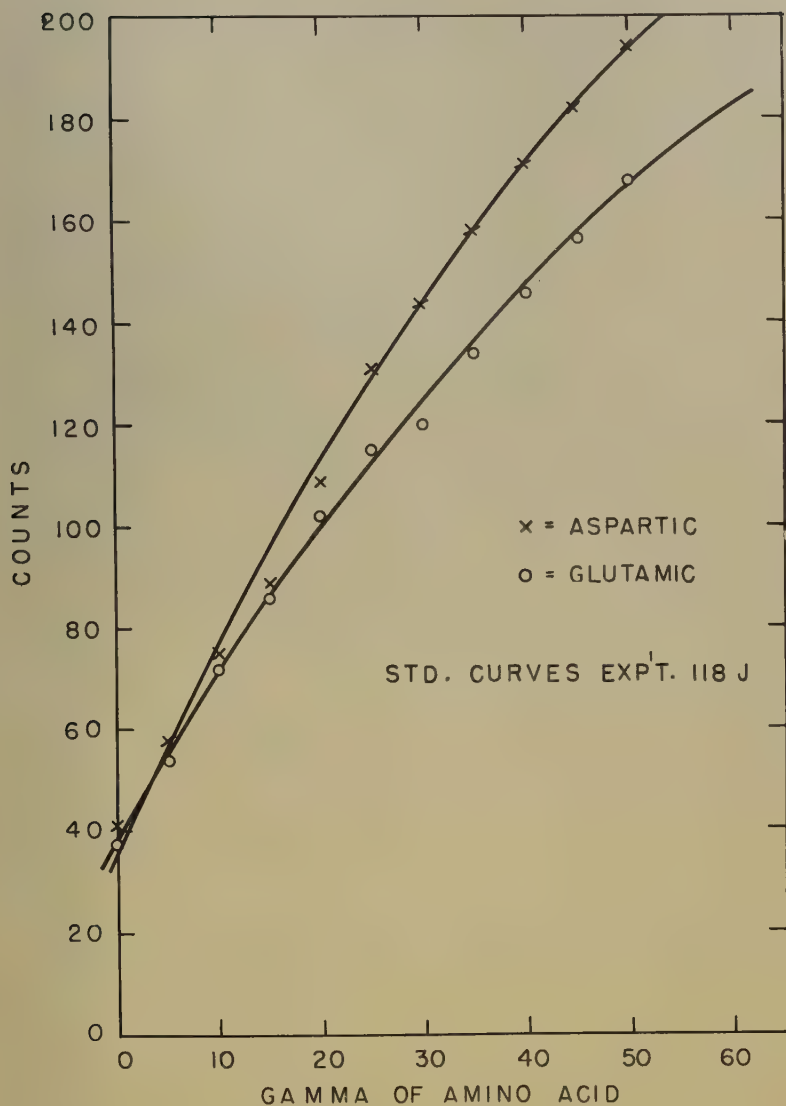
FIG. 2.—Typical standard curves given by glutamic and aspartic acids.
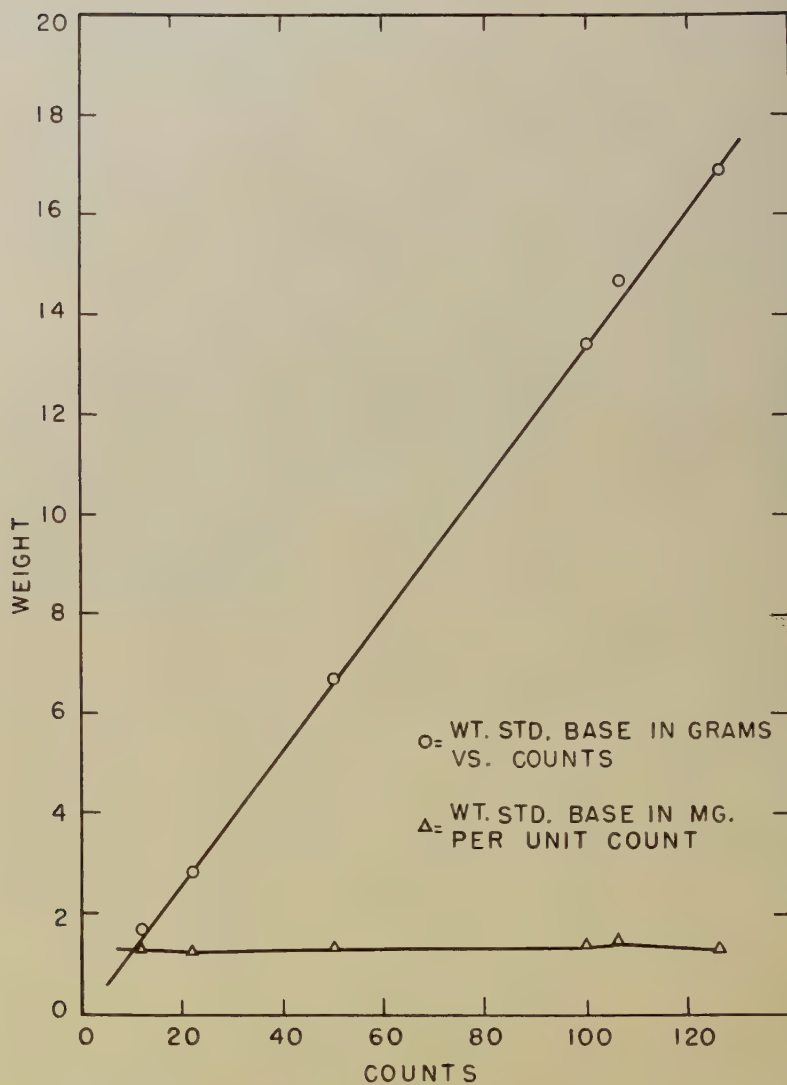
FIG. 3.—Weight of standard base delivered by dispenser.

TABLE 2

WEIGHT DELIVERED BY DISPENSER FOR TEN RANDOM SAMPLES

| Tube No....... | 9 | 89 | 10 | 13 | 8 | 0 | 16 | 12 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|
| Wt. delivered... | 3.34 | 3.34 | 3.35 | 3.32 | 3.36 | 3.34 | 3.37 | 3.36 | 3.36 | 3.38 |
| Av. wt. delivered.... | | | | | 3.35 | | | | | |
| Range of wts. delivered.... | | | | | 3.32–3.38 gms. | | | | | |

\* Tubes were filled at the rate of 10 tubes per minute.

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